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A COMPARATIVE STUDY OF CONIDIAL FORMATION IN CEPHALOSPORIUM AND SOME RELATED HYPHOMYCETES[1]

M. ELIZABETH PINKERTON
Formerly Graduate Scholar in the Henry Shaw School of Botany of Washington University

I. INTRODUCTION

Among the organisms isolated by the author from dermatomycoses at the Barnard Free Skin and Cancer Hospital, St. Louis, Missouri, a red yeast, identified as *Torulopsis mucilaginosa,* appeared frequently. On ordinary media it formed a rose-red, pasty, and superficial colony; in liquid, a basal deposit and some pseudomycelial proliferation throughout the solution. Upon nitrate agar of Stellung-Dekker ('31) the growth was homogenous and scanty on the surface and cloudy-white within the medium. Microscopic examination revealed a pseudomycelium bearing numerous lateral spore balls reminiscent of *Cephalosporium* (pl. 2, fig. 43). This suggested a further study of the cause of the deviation and the relationship of the budding and cephalosporial states. A cursory investigation of the pertinent literature disclosed much confusion regarding the types of Hyphomycetes which form cephalosporia (spore balls). Hence, the problem has been extended to a comparative morphological study involving principally

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forms which may grow parasitically or saprophytically upon the human skin.

It is the primary aim of this dissertation to explain the mechanism of cephalosporial production and to correlate such findings with data on other types of spore-producing organs. Observations of the pathogenicity of these organisms for man are recorded. Also, on the basis of methods of production and kinds of spores, as well as upon other physiological and morphological observations, the group is discussed systematically. Present treatments are criticized and emended, at least in consideration of the few forms studied.

II. MATERIALS AND METHODS

Investigations have been made upon the following species. For further discussion of systematics see p. 37.

ORGANISM	PLACE OBTAINED
Allescheria Boydii Shear	Centraalbureau voor Schimmelcultures, Baarn
Cephalosporium kiliense (Gruetz) Hartmann	Centraalbureau voor Schimmelcultures, Baarn
Cephalosporium niveolanosum Benedek	Centraalbureau voor Schimmelcultures, Baarn
Cephalosporium rubrobrunneum Nannizzi	Centraalbureau voor Schimmelcultures, Baarn
Cephalosporium Serrae Maffei	Centraalbureau voor Schimmelcultures, Baarn
Cephalosporium Stuehmeri Schmidt & Beyma	Centraalbureau voor Schimmelcultures, Baarn
Cephalosporium symbioticum Pinkerton	Barnard Free Skin and Cancer Hospital, St. Louis
Clonostachys Araucaria Corda	Centraalbureau voor Schimmelcultures, Baarn
Clonostachys Araucaria var. *rosea* Preuss	Centraalbureau voor Schimmelcultures, Baarn
Clonostachys Araucaria var. *confusa* Pinkerton	C. Thom
Clonostachys spectabilis (Harz) Oudem. & Sacc.	Centraalbureau voor Schimmelcultures, Baarn
Gliocladium deliquescens Sopp	C. Thom
Gliocladium fimbriatum Gilman & Abbott	C. Thom
Gliocladium penicilloides Corda	C. Thom
Gliocladium Vermoeseni (Biourge) Thom	C. Thom
Scopulariopsis brevicaulis (Sacc.) Bainier	C. Thom
Torulopsis mucilaginosa (Jörgensen) Ciferri & Redaelli	Barnard Free Skin and Cancer Hospital, St. Louis

The stock medium was glycerine agar (nutrient agar plus 6 per cent glycerine, pH 6.9). The following agars were also used: maltose (pH 5.4), Sabouraud's dextrose (pH 5.6), wort (pH 4.8), Raulin's (pH 4.15), Czapek's (pH 4.43), Richards' (pH 4.36), Endo's (pH 7.52), Gorodkova-Maneval modified (pH 6.8), nitrate (pH 5.5), nutrient (pH 6.0), corn meal (pH 6.0), potato dextrose (pH 5.7); these liquids—nutrient (pH 6.0), lactose (pH 6.8), litmus milk; also sterilized carrot plugs, gypsum blocks, Bacto-beef blood serum (pH 7.3), and glycerine agar double strength plus 50 per cent Bacto-blood (pH 6.0). The latter gave the most luxuriant and rapid growth.

Cultures were made chiefly in test-tubes and bits of them were examined at intervals in semi-permanent mounts of Amann's ('96) lacto-phenol plus a 1 per cent aqueous solution of anilin blue. Such served for gross morphology and even showed a fair degree of the more minute structure of the phialide. The proper relationships of the various parts of the organism and their comparative colonial characteristics are shown in the photographs of Petri plate cultures containing Czapek's agar (pl. 6). Cultures were also examined directly under high power after placing a cover slip over a sparsely grown section of the agar. Camera-lucida sketches were made of relevant structures and photomicrographs were taken where possible, but due to the minuteness of the conidial fructifications the latter procedure was not very practical.

Hanging drop and agar preparations made in van Tieghem cells were of little use, since the fruiting bodies were produced pendant in the air and hence not discernible under oil. Agar slide cultures, produced by placing a few drops of warm agar upon a sterile slide, inoculating, and applying an aseptic cover slip (No. 1, 24 × 40) when the agar was cool enough to barely spread, were found to be most helpful, giving a comparatively small circular agar matrix from which aerial fructifications radiated. The narrow ends of the cover slip were sealed with paraffin to make the mount rigid, and the microcultures incubated at room temperatures in sterile staining jars with moist filter-paper at the bottom. On the second day the formation of spores could sometimes be observed under oil immersion.

Material for cytological study was grown on Raulin's agar and on glycerine agar. Hermann's fluid[1] was employed for killing and Jeffrey's celloidin technique was followed for the earlier work. The fixing agent was applied directly to the slant culture which was evacuated for about fifteen minutes to remove air from the agar containing submerged growth. After twelve hours the material was washed for an equal time in slowly running water, following which the agar was cut into small blocks about 5 mm. square and run through the alcohols.

Since it was impossible to get celloidin sections thinner than 10 μ, for studying structures of 2 μ average diameter the paraffin method was tried. After the agar culture had been cut into small bits, good infiltration was secured and sections were obtained 1, 3, 5, 8, and 10 μ in thickness. The very thin ribbons had the disadvantage that so few aerial phialides were encountered in a slice. Nevertheless, nuclear orientation was clearer in the paraffin material, whereas accessory cytoplasmic inclusions and external mucoid structure were more noticeable in the celloidin matrix. Haidenhain's iron-alum haematoxylin was used exclusively for staining, applied according to the following schedule: hydration, one hour in 20 per cent USP hydrogen peroxide, washing, 4 per cent iron alum two hours, ½ per cent haematoxylin twelve hours, slight destaining with 2 per cent iron-alum while watching through the microscope, dehydration, clearing in xylol, and mounting in Canada balsam. The species cytologically investigated include: *Allescheria Boydii, Clonostachys Araucaria, C. Araucaria confusa, Cephalosporium Serrae, Gliocladium deliquescens,* and *G. penicilloides.*

An attempt was made to compare the wall composition of the ordinary mycelium and of the phialide by use of the polarizing microscope. The thinness of the cell walls and the difficulty of obtaining sufficient light with the higher magnifications prevented satisfactory observation. Hanging drop cultures, where the fructifications could be brought into focus, served best.

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[1] The formula is: platinic chloride 3 cc. in 12 cc. of distilled water; osmic acid 2 cc.; glacial acetic acid 1 cc. A fresh solution should be used for each application.

All of the organisms were grown at 30° C. The cultures were usually incubated in the light, since darkness often caused loss of color without other apparent changes. In an attempt to induce the perfect stage, glycerine agar was employed with H-ion concentrations from 3.8 to 7.6; and the following special media were utilized: gypsum blocks, Gorodkova-Maneval medium, carrot plugs, and corn meal agar. Attempts at cross fertilization were made, and while no perfect states were induced some interesting subsidiary observations were obtained.

III. The Conidial Apparatus

A. THE CONIDIOPHORE

The forms here investigated are believed to be closely related to *Penicillium* of the sub-family Aspergilleae in the Hyphomycetes. The form-genus finds its perfect stage in *Carpenteles* Langeron ('22) of the ascomycetous order Plectascales, although the latter name is by no means in common usage. Its conidial apparatus consists of a basal stalk cell from which branches arise variously but more or less regularly for each species. The end cell of the penicillus, called the phialide, cuts off spores terminally in chains. The spores are temporarily adherent, due to connectives which later become disjunctors and probably are formed from the old wall of the phialide. In the material of the present study there are no disjunctors, and the spores mutually adhere in a more or less globular mucilaginous mass around the phialide. The spore ball is termed the cephalosporium in contrast to the entire fructification, or cephalophore. In most cases the cephalosporia are characteristic of the aerial mycelia, although submerged ones have been noted in *Cephalosporium Serrae.* The complete conidiophore varies from a short branch no longer than the spores produced (e. g. *Allescheria Boydii*) to a complex structure equivalent to the penicillus (e. g. *Gliocladium*); but in all cases where cephalosporia are produced the mechanism seems identical.

1. HISTORICAL

a. On conidial production in the Aspergilleae and related forms.—Brefeld ('74) was one of the earliest to observe critically conidiophore formation although his methods were necessarily crude. He saw no need for the assumption of a special membrane outside that of the conidium, and thought that the process was one of budding. Referring to the forms with disjunctors, Seynes ('86) stated that the phialide wall formed the connective and the conidia differentiated their own walls within. The following statement of de Bary's ('84) exhibited keen insight although his interpretation was incorrect:

> Bei reichlich inner halb enger und mit enger Mündung versehener Behälter abgeschnürten Sporen wird durch die Gallert- oder Gummiabscheidung die Entleerung aus dem Behälter bewirkt, indem die durch Wasseraufnahme quellende Masse aus der Mündung hervortritt. [p. 75].

He believed that there was a gelatinous substance around the spore (a product of its outer lamella) which became deliquescent by addition of moisture and so caused coherence.

A number of investigators attempted to interpret the method of spore production in *Thielavia basicola* of the Plectascales, following Zopf's original description in 1890. He reported an acropetal succession of spores whose lateral walls were differentiated into two layers, the outer being a sheath through which the conidia emerged successively due to the swelling of the mucilaginous middle lamella upon the addition of water. Gilbert ('09) thought that endoconidia were formed in the phialides and emitted consecutively by the force of the growing protoplasm in the basal part of the cell. Brierley ('15) was the first to make a definite attempt to determine the method of conidium production, and he concluded that the method previously called endoconidial was in reality merely acrogenous, being peculiar only in the mechanism of liberation. The conidium put down an additional wall within the parental one which was then ruptured and the new spores were constantly formed from below. The illustrations of the mode of new wall differentiation are quite anomalous. It was Duggar's view ('09) that the endoconidia were ejected by osmotic pressure. Guegen ('05) cited *Gliomastix chartarum* as an example of endoconidial production.

Buchanan ('11) thought that the spores of the cephalosporium were formed by the abstriction of the tip of the sporophore, the amount of moisture in the air apparently conditioning the amount of adherence. He regarded as unnecessary the separation of *Hyalopus* and *Cephalosporium* upon the basis of more slime in the former. Studies of *Cephalosporium* spp. on insects led Petch ('28) to agree with Buchanan that the spores were really cut off in succession but became aggregated by the secreted mucilage. In a damp atmosphere some were found in loose chains due to the dissolution of the mucilage. He too disregarded the separation of *Hyalopus* and *Cephalosporium.*

In *Penicillium digitatum* Scaramella ('28) illustrated the rupture of the parent sheath anew below each successive spore. Thom ('10) thought that the spores were merely acrogenous disjunctions which produced their own internal walls and were held together by the phialide wall which did not necessarily rupture until late. This would seem to indicate continued elasticity of the conidiophore wall below the tip. In 1930 he added the following hypothesis regarding submerged cephalosporia:

> . . . that the impulse to produce conidia when acting in a submerged hypha in which all parts are in constant contact with the nutrient, produces a transformed branch bearing spores which tend to enlarge, round up as if for germination, and to lose the catenulate arrangement. This may produce a very peculiar spore attached as a single terminal cell or a cluster of cells not recognizable as conidia and without definite arrangement around the tip of the sterigmatic cell. [p. 73].

Wakyama ('31), in cytological investigations upon *Aspergillus* spp., indicated that the daughter nucleus migrated through a sterigma very similar to the condition in the basidiomycetes, but he did not further investigate the mechanism. Finally Dodge ('35) suggested that the mechanism of conidium formation in the genus *Cephalosporium* is probably close to that of *Aspergillus* and *Penicillium* although the conidiophore is not flask-shaped. If the spores are essentially in chains, they slip past one another very soon to form spore balls.

b. Previous interpretations of the mechanics of spore production in general.—There are probably more common factors in all types of spore production than is generally realized, because the cutting out of similar small masses of protoplasm would not have many physical possibilities of variation. There have been numerous papers regarding this process in the sporangia of the Phycomycetes, a few on ascospore delimitation, and a number on the basidiospore release; whereas, except for Brierley's work, the conidia have generally been neglected.

There are a number of forces which have been championed for spore formation: vacuolation associated with exudation of water or some other liquid; centrosomes or similar bodies which are centers of force and direct the action of protoplasmic streaming; surface tension; Golgi apparatus or the like associated with secretion; and electric forces.

VACUOLES AND EXUDATIONS.—Harper ('99) saw in the sporangium of *Synchytrium decipiens* an early shrinking of protoplasm probably associated with a throwing off of water which accumulated in vacuoles wherein cleavage furrows arose simultaneously throughout the sporangium. In certain cases uninucleate masses were separated, in others multinucleate. He explained the inclusion of a nucleus by the characteristic chemical contents of nucleo-proteins and nucleic acids which were centers of moisture retention or at least had a greater affinity for water than the surrounding cytoplasm.

DeBary ('84) attributed the bursting of the ascus to increased vacuolization due to the absorption of water. In the cutting out of the sporangiospore segments of the Phycomycetes, he mentioned delimiting granular plates which somehow became gelified.

Exuded droplets were shown by Buller ('22) to be significant in the disjunction of basidiospores in all Basidiomycetes except the Gasteromycetes and the Ustilaginaceae. He found droplets also associated with the conidia of the Hymenomycetes and with the aeciospores of the rusts, but thought that such were merely passive. It is possible in these cases too, that, although not effecting violent spore disjunction, exudates may somehow bring about their release. In *Coprinus sterquilinus* he noted that a drop of water 5 μ in diameter appeared at the base of the basidiospore within ten seconds before its abjunction, and that the liquid was shot off with the spore and doubtless aided in attaching it to the substratum. It was supposed that the liquid came from the hilum, and was largely water, rather than local mucilagination from the sterigmata (often so narrow as 0.5 μ). The latter, however, must have been ruptured, for it soon collapsed. Buller regarded the production of similar drops in *Sporobolomyces* as suggestive evidence that the genus belonged to the Basidiomycetes in spite of the reduced vegetative growth and completely uninucleate state (Guilliermond, '27). However, Stempell ('35) believed that the water-drop mechanism was merely an ecological response and not of phylogenetic importance. He reported its occurrence in association with both the sporidia and secondary conidia of *Entyloma* of the Tilletiaceae, and also with the sprout cells of *Taphrina,* usually included in the Ascomycetes.

Buller thinks that the hydrostatic pressure of the basidial vacuole is very important in forcing the protoplasm and nucleus through the narrow sterigmata and in the final spore discharge, since the basidium collapsed immediately after the final release of one set of spores: "Now my experience, based upon extensive observations on the living hymenium, is that a basidium never produces more than one generation of spores and that after producing a single crop of spores, its sterigmata and body quickly collapse." [Buller, *l. c.* **2**: 28.]

CENTROSOMES, SURFACE TENSION, AND GOLGI APPARATUS.—Maire's ('02) illustration of basidial development in *Psathyrella disseminata* first suggested a centrosomal interpretation of the terminal granules in the imperfects studied. The four centrosomes in the former, produced simultaneously with the four nuclei, situated themselves at the apex and followed the protruding sterigmata with tails of streaming protoplasm ("cordons kinoplasmiques") eventually drawing the nuclei into the spores. These structures, formerly believed to be confined to fungi, algae, and animal cells, are now being discovered frequently in the higher plants. They are apparently centers of energy associated with the action of the spindle in the separation of chromosomes, as well as with the movement of flagella and cilia, and are generally believed to be absent in the resting cells. There has been controversy over their origin —cytoplasmic or nuclear—and confusion of them with other structures.

Faull ('04) concluded that the centrosomes were of extranuclear origin and occurred after reforming of the membrane. Guilliermond, Mangenot, and Plantefol ('33) stated that the centrosome was still an enigma and if it aided in ascospore delimitation, its influence was purely physical, perhaps by the superficial tension on the surface of the asteral parasol which conditioned the formation of new walls. Harper ('99) firmly believed that the aster rays (fibrous kinoplasmic elements) cut out the ascospores and formed the limiting walls. They might well direct the spore delimitation but were themselves included within a special wall, according to Faull ('04). Surface tension and perhaps a different chemical composition kept the endospores discrete from the epiplasm.

Buller ('09), in discussing surface tension and the physics of the jet theory of the ascus, stated that a free cylinder of any liquid under the influence of surface tension became unstable as soon as the length was greater than πD. It is a necessary consequence that if once realized the diameter of such a cylinder will divide into as many equidistant spore lengths as πD is contained in one length. Some such physical interpretation must be necessary for the regulation of the size of the endoconidial segments in the forms studied by the author.

Scarth ('27) thought that a definite structural basis of protoplasm was necessary for life movements. He observed a reciprocity of substance in the increase of mitochondrial elements correlated with the disappearance of the active kinoplasm as a result of vital staining; and concluded that the characteristic component of the chondriome, viz. lecithin, entered into the composition of the kinoplasm, and that the mitochondria merely served as reserve substances for the formation of the more labile elements. Vacuoles were thought to arise from the kinoplasm by the rounding up of fibrillae or as blisters or bubbles from the covering films of the chloroplasts or of the central vacuole. The wandering streams of protoplasm in the cytoplasm condensed into mucilaginous-looking globules which distended as vacuoles; and an essentially similar process effected the cleavage of coenocytic sporangia. In some cases, however, fissuring arose from fibers that radiated from a central body attached to the nucleus, in others by invaginations of the ectoplasm or by outgrowth of vacuoles. The limiting film of these extensions of the kinoplasm became the plasma membrane of the spores, while the fluid which was secreted between them was presumably analogous to the sap which formed in the vacuole.

Sharp ('34) regarded the divisions by constriction, seen in unicellular algae, in the budding of yeast cells, and in the abstriction of conidia and basidiospores, as special cases of cytokinesis by furrowing. The centriole, or inside of the centrosome, has been generally believed to be a point of high surface tension, and the aster, according to Sharp, might be primarily an expression of streaming movements in the cytoplasm which is somewhat more solid than the rest of the more gelified granular protoplasm. He further said that in the plant cell the furrows probably separated regions of relatively high viscosity even though no aster, in the ordinary sense, was present: "Alterations in surface tension and viscosity, together with protoplasmic streaming, are obviously important factors in cytokinesis of certain types, but comparatively little is known about the initial causes of these phenomena."

Wilson ('25) mentioned the appearance in epithelial muscular and glandular cells of a chromidial apparatus assumed to be extruded from the nucleus and of particular function in trophic actions of the cell. He also thought that there was a definite karyoplasmic ratio which regulated cleavage to produce a fixed or typical cell size at a given stage rather than a fixed number of cells. It was the surface of the nucleus and not its volume that was directly proportional to the number of chromosomes.

The Golgi apparatus is known only in animal cells and is connected with secretion. In goblet cells of the colon, secretory granules have been said to have originated in mitochondrial (chondriosomal) regions and to have migrated to the Golgi region where they were transformed into mucin granules (Duthie, '33). The granules were then liberated into the cytoplasm, finally dissolved, and passed as liquid secretion into the lumen. This apparatus has also been suggested as a center for the formation of enzymes. Whether a similar process occurs in plants with liberation of mucin has not been investigated. The function and limitations of mitochondria are also disputed questions. Guilliermond ('29) homologized the plant vacuome —which he thought arose *de novo* from the cytoplasm—with the Golgi apparatus and Holmgren canals of animals. Nassonov ('23) believed, on the basis of staining experiments, that the vacuolar membrane had a secretory function and was homologous to the Golgi apparatus.

Weier ('32) thought that the young plastid in spermatogenous, archesporial, and epidermal cells of *Polytrichum commune* and young androcytes of *Pellia epiphylla* showed staining similarities to the animal Golgi apparatus and believed that such might be associated with enzyme secretion, in the former case linked with starch production and in the latter with secretory granules. He did not think, however, that the two should as yet be considered homologous.

Sass ('34) offered further evidence of a Golgi apparatus in the Basidiomycetes, although he failed to associate it with any property of motility as in animals or with the plastidome reported by Weier. The *"nebenkern"* in *Coprinus sterquilinus* was found to divide into four "Golgi crests" which eventually became situated at the tip and presumably cooperated in the formation of sterigmata and in nuclear migrations. These elements resemble superficially the centrosomes of Maire ('02). His results were especially interesting in that he used various new fixatives absolutely free of osmic acid, which eliminated possible errors due to diverse osmophilic substances.

2. INVESTIGATIONAL RESULTS

Because in all cases investigated the mechanism of spore formation has appeared to be similar, the results shall be outlined in general for the whole group. Plate 1 includes exemplary types selected at random and the figures here mentioned refer to that plate.

The incipient phialide shows a blunt tip which is thicker than the lateral walls and stains darker, be it typically flask-shaped as in *Gliocladium fimbriatum* or essentially straight as in *Cephalosporium niveolanosum.* The dense granular contents do not extend quite to this tip since there is a hyaline area between (fig. 2). A chain of small round granules collect longtitudinally, and in fixed preparations show "tails," suggesting rapidly streaming protoplasm (fig. 3). These granules apparently merge into several small rods (fig. 4). Presumably at about this stage a drop of liquid is exuded terminally (figs. 5 and 6); at least in living cultures on hanging drops and agar slides these drops are infrequently noted on blunt hyphae. In the latter material the globule sometimes disappears as it is viewed and runs down the side of the phialide, producing the obviously different appearance of this structure, so often noted, as compared to the rest of the mycelium. In the hanging drops, with low magnifications the downward-hanging fructifications portray cephalosporia with spores literally swimming in the slime which here has not been disseminated by touching the glass. In the next step (fig. 7) a cross-section of the tip shows three granular rods, horizontally aligned, from each of which is a narrow short strip of cytoplasm leading to the protoplast proper. Figure 8 shows a split between four rods which "hold open the sac." The blunt tips seem to be especially resistant at the corners, and the adherence of rods to the sides probably increases this opposition so that the tip swells in the center slightly before splitting.

In the meantime, presumably associated with the exudation of liquid, the contents of the phialide have cleaved into a varying number of segments which at first are noted as regularly occurring cross-lines of granular dark-staining material, indicating regions of high surface tension. Later, narrow hyaline areas appear between the segments which round up at the edges. At the very base of the phialide are a varying number of small vacuoles. The spore, clad only in a fine membrane, begins to protrude from the rigid collar of the phialide whose narrowness considerably retards the process (figs. 9 and 16). As if forced out by pressure from the increasing vacuoles at the base, the spore becomes somewhat constricted, as indicated by its tendency to round up on emergence, and causes the included end to become increasingly convex. Finally, after the greater part has emerged, the rest of the spore snaps off, and is immediately followed by a successor. This process is relatively slow, under optimum conditions only three spores being emitted from a phialide during seventy minutes (fig. 17). The exuded slime and the moist surface of the spore hold it near the phialide. In the spore ball it cannot escape until moisture disseminates the mucilage. Very thin sections of paraffin material seem to indicate that the spore is abstricted as is the yeast bud (fig. 15). This appearance may be explained by assuming that one of the granules at the tip really stays in the center of the phialide and draws the cytoplasm as a narrow stream into the spore; and in some thin sections it is impossible to see any lateral granules. Moreover, young phialides show blackish regions at the tips which probably represent central granules.

Since the greatest diameter of the phialides averages only 2 μ and the contents are granular, it is impossible to make out nuclei in the celloidin material, but in the thinner paraffin sections each cell and endoconidial segment appear to be uninucleate. The energid is but slightly larger than other dark-staining metachromatic granules throughout the cytoplasm. No definite mitotic stages were espied. The fine stream of protoplasm passing into the spore stains very darkly, perhaps due to a passing nucleus or to compression and rapid streaming. Sometimes mature spores show numerous refractile granules, at other times a more definite accumulation which is regarded as a true nucleus. After emergence, the spores form a thin wall around the membrane, swell doubly or triply, and germinate after several days. In some cases they appear to be germinating while still within the ball (pl. 5, fig. 16). Since the mucilage swells upon the addition of water, when favorable moisture conditions occur the spores are released both for dispersal and germination. In the meantime, they have been protected by the slime. Thus cytologically the conidia in these species are haploid representatives of haploid individuals, apparently capable of indefinite existence without fertilization.

While the spores are being released the vacuolar area within the phialide multiplies conspicuously. The latter is increasingly contorted and devoid of protoplasm (especially noticeable in the larger single conidiophores of *Clonostachys Araucaria*). Doubtless, the vacuolar force, as well as the mechanical one of twisting, aids in the liberation of the spores. Neither by differential staining nor polarized light were any essential differences in the chemical nature of the phialide and ordinary mycelium observed.

Young tips of ordinary hyphae were more clearly observed in hanging drops than on agar. They are always more or less pointed and of the same consistency as the rest of the wall. Vacuoles extend quite close to the apex and contain one or several very active and large dancing bodies (pl. 3, fig. 21). Buller ('33) mentioned highly refractive particles (Woronin bodies) in *Ascobolus pulcherrimus,* on both sides of the septa and in terminal cells in a dispersed condition. In older cells they were said to settle down in the walls of the vacuoles, never in the protoplasm proper, and were associated always with the sap cavity, even passing through septa with it. These may be identical with the large dancing bodies, or even the larger septal granules (pl. 3, fig. 20), although the author does not think the latter two are the same. In fact, there seem to be dancing bodies which appear dark even *in vivo,* and cytoplasmic granules which show only in fixed preparations. The crowded vacuoles with small metachromatic granules visible between them soon occupy approximately the whole cell except for a narrow peripheral layer and a cytoplasmic region in the center where the nucleus is hung (pl. 3, fig. 19). Around all aerial hyphae on agar slides was noted a fine layer of mucilage, which indicates that apparently such exudation is normal in the life of the organism, and differs only in quantity in the phialide.

For comparison, a cursory examination was made of living material of *Scopulariopsis brevicaulis* (Sacc.) Bainier, a species whose spores are held together in chains by disjunctors. The process agrees very well with that shown by Scaramella ('28) in *Penicillium digitatum* Sacc. The terminal uninucleate segment puts down an internal wall as a chlamydospore does, and by its swelling ruptures the external wall at the shortest diameter of the ellipsoidal spore which remains adherent at the base and connected with the rest of the phialide. This leaves empty a lens-shaped connective between the chain of spores abjointed similarly and successively. Meanwhile, there has been a slight cleavage of the protoplasm into a number of segments within the phialide, so that although only one spore becomes encysted at a time the potential ones are already present as in the phialide of the cephalospore. The main differences in the latter are the lack of resistant spore walls and method of rupture of the phialide, whose functions, as well as that of the disjunctor, are taken over by the mucilage. Comparative cytological data would be interesting.

3. INTERPRETATION

It seems probable that the incipient phialide eventually forms a rigid tip which is no longer capable of stretching and of intussuscepting material. The contents of the cell are continually swelling due to vacuolar as well as osmotic pressure and to surface tension, indicated by accumulation of granular material which eventually causes a rupture. In the meantime the granules have strengthened the corners so that the middle of the tip is stretched and broken and the size of the opening is considerably narrowed. The exudation of the mucilage, whether effected by the granules or not (though such seems likely, and it must come from the living protoplasm itself), doubtless makes possible the cleaving of the protoplasm as has been suggested in the basidium and sporangium. Whether the terminal granules represent centrosomes or Golgi bodies, the author is not prepared to state; but at any rate they must represent kinoplasmic elements similar to those of Scarth ('27). They may be an aggregation of chondriosomes whose significance is not clear.

Further, the mucilage production may be compared with guttation in the angiosperms (Wilson, '23), where there were found 220–1030 ppm. of organic material, mostly nitrates and nitrites, in the exudate. This indicates that occasionally colloidal material can be passed through intact walls. The terminal wall was not broken in the phialide at first, since it was sometimes visible within the droplet. The theories that the slime arises from between the membranes, as suggested by Knoll ('12) in cystidia, or by deliquescence of the middle lamellae (Brierley, '15) or of spore walls themselves (deBary, '84), are plainly inapplicable here.

Granules such as those occurring terminally in phialides are not noted in the ordinary mycelial tips, although similar (?) ones (pl. 3, fig. 20) are discernible in ordinary cross septa, perhaps exercising a function associated with the protoplasmic connectives.

The cleavage itself is largely a surface tension phenomenon and is doubtless often only partial, i. e., the cleaved parts occur only in small numbers towards the tip of the phialide and this process may be repeated; hence all the nuclear divisions do not have to take place at once. Such is exemplified in embryological studies of cleavage of animal eggs. Possibly, due to the water-holding capacity of the nucleus, liquid is exuded equidistant between the energids where increased surface tension is noticeable in fixed material by a layer of basophilic granules. Buller's physical theory would explain the size of the segments (cf. p. 10 of the present paper). The spores are released by the vacuolar pressure in the lower part of the phialide. Cohesion and surface tension factors, associated with a narrow rigid opening and the tendency of an elastic portion of a spore to round up when the pressure is released, assist the process. The size and shape of the spore are doubtless dependent upon the extent of the phialide and its opening as well as upon the amount of cytoplasm included in the endoconidium, all being explainable upon a purely physical basis. That the species of *Cephalosporium* have the longest narrowest spores is due to a comparatively straighter phialide which makes the endoconidial column more extensive. Extrusion thus takes longer, and the membrane or new cell wall becomes more or less rigid before the spore is completely released.

The purely physical force of pressure must also be assisted by that of twisting caused perhaps by spiral streaming of the protoplasm. Although the author has seen no previous mention that this force assists in spore dispersal, Castle's ('34) reference to it in growth is suggestive. He states that the twisted or helical forms of growth are generally due to the resolution of two growth vectors: one longitudinal and one rotational. Oort ('31) had measured simultaneous growth and twisting along the axis in the coenocytic spore-bearing cells of *Phycomyces* and found at 17.5° C. an average rate of elongation of 39 μ a minute and an average rate of rotation of 3.7 degrees a minute. The angle at which micelles were incorporated in the wall was about 6 degrees. The steepness of the growth spiral was not structurally fixed but was reversibly altered (reversed or abolished) by change of temperature. The majority of the cells grew in the form of a right-handed helix, i. e., left-handed thread on a screw. The rapidity with which the angle of coiling changed seemed to refute the interpretation of altered proportions of different types of isomeric molecules. It may be that spiral streaming of protoplasm helps in the phialide twisting, but this is purely conjectural since no proof has been obtained.

The eventual collapse of the phialide results from the lack of compensating turgor pressure from the end wall which has become open (also an aid in the spiral twisting). Such a condition is less noticeable in the typical penicillate fructifications which have the most minute openings, especially in comparison with those of the rest of the phialide, than in the broader, straighter, and simple ones of *Clonostachys Araucaria.* If the spores were merely produced acrogenously there would be no exhausted remnant; moreover the endogenous condition here is more similar to that in the basidium and less like that in the ascus because there is no residual epiplasm.

The slime forms a protective covering around the delicate thin-walled spores until optimum moisture conditions arise, when it not only makes the spores available for germination but also causes considerable dispersal due to the colloidal swelling accompanying its solution in water. Because of this miscible property the amount persistent around the spores will depend upon the humidity of the atmosphere. In very dry conditions the external layers tend to become almost rubbery, as ascertained by Cooke and Ellis ('78) in the solid slime balls on herbarium specimens of *Penicillium repens.*

The submerged cephalosporia of *Cephalosporium Serrae* appear to be homologous with the aerial ones except possibly for a greater tendency to irregularity in shape due to the confining action of the medium. In *Torulopsis mucilaginosa,* on the other hand, the whole growth is obviously a budding pseudomycelium and actual production of endoconidia was not observed. In *Cephalosporium symbioticum,* the cephalosporia attached to the short laterals more nearly simulate the fructification of *Allescheria Boydii* than that of *Cephalosporium.* Since this condition occurs in *Torulopsis mucilaginosa* only in submerged growth, it is an abnormality, and whether it may indicate a foreshadowing of the conditions in the regular forms or a reversion from an advanced to a reduced type (the yeast) is still a question. The latter view is the more logical.

In agreement with Wakayama ('31), the present data indicate that the conidiophore is an asexual organ of the haplont producing uninucleate spores which repeat the life cycle without variation except for secondary asexual spore forms. The occasional conjugation of conidia is without significance so far as is known.

B. OTHER CONIDIAL FORMS ENCOUNTERED

Omnipresent in this group are terminal or intercalary chlamydospores whose new cell walls are put down inside the parent cells. The production of chlamydospores is ordinarily associated with adverse conditions, and dissemination occurs when the adjacent cells die. Besides these, in *Gliocladium fimbriatum* and *G. deliquescens* large terminal cells rich in oil droplets are formed, which differ from chlamydospores in their relatively thinner walls and capability of immediate germination as evidenced in hanging drop cultures. For such, the designation akinete has been adopted from algological terminology. Moreover, there are noted within the medium enlarged round to ovoid terminal cells beyond which the hypha may grow again (pl. 3, fig. 30). Their subsequent development was not followed, but when observed they were thin-walled. They may possibly be aborted ascogonia, and Thom has referred to them as due to the same impulse as that causing submerged cephalosporia. If they function as chlamydospores, at least their method of formation is somewhat irregular.

The peculiar short-stalked spores produced promiscuously over the terminal clubs in *Clonostachys spectabilis* cannot be discussed in detail since the culture died early, but they are obviously not comparable to the other forms studied. A superficial resemblance between spores and the warty structures of the phialides of *Clonostachys Araucaria* may have caused Oudemans ('86) to include that species in *Clonostachys*. However, the warts are not spores but thickenings on the wall, not uncommonly seen in fungi and are incapable of germination.

Infrequently oidia were noted in *Gliocladium Vermoeseni* (pl. 4, fig. 11). The significance of these secondary spore forms is obscure. If such occur in *Penicillium* and *Aspergillus*, Thom ('26, '30) did not mention them, unless the "hülle" cells first cited by Eidam ('83) in some species of *Aspergillus* could be considered such. It is obvious that hypnosporic types of spores are produced in response to unfavorable conditions, especially where the colony has exhausted the medium and an indefinitely resting spore is needed.

IV. PHYSIOLOGY

A. COMPARATIVE CULTURAL CHARACTERISTICS

In table 1 are listed most of the organisms studied on a representative number of media, with brief notes regarding color, surface, and microscopic characteristics. Some correlations

TABLE I

COMPARATIVE CULTURAL CHARACTERISTICS IN VARIOUS MEDIA

Organism	Nitrate pH 5.5	Czapek pH 4.43	Potato dextrose pH 5.7	Corn meal pH 6.0	Gorodkova-Maneval pH 6.8	Sabouraud's dextrose pH 5.6	Lactose broth pH 6.8	Sabouraud's Conservation pH 7.0	Glycerine pH 6.9	Carrot plug
Allescheria Boydii	Gull-gray, powdery, submerged; single spores.	Gray-white, flat aerial.	Gray-green, black below, wrinkled; coremia; single spores.	Gray, powdery; single spores.	Tan-gray, flat, matted, filamentous; single spores.	Pale-gray, long, fuzzy; many single spores.	Gray pellicle of small radiating colonies.	Gray-violet, black, flat, powdery.	Dark gray, corrugated; single spores.	Gray-green, short, fuzzy.
Cephalosporium kiliense	Apricot-buff, cerebriform; chlamydospores; submerged cephalosporia.	Orange, rough, heaped, edges submerged.	Deep orange, furrowed; cephalosporia; chlamydospores.	Pale pink; submerged chlamydospores and cephalosporia.	Pale pink, flat, moist, sterile.	Pink, corrugated; coremia; small cephalosporia.	Pink ring, liquid turbid.	Pink, moist, much wrinkled.	Strawberry-pink, slightly wrinkled; many coremia.	Pink, tallowy, with radiating coremia.
C. niveolanosum	Shrimp-pink, flat, wrinkled; chlamydospores; submerged cephalosporia; long coremia.	Bluish-white, flat, with radiate striations.	Yellow-white, heaped in center, hairy projections, sterile.	White, wrinkled; many cephalosporia.	Pale tan, heaped, hairy protrusions; chlamydospores.	Few cephalosporia.	White yeasty ring, slight turbidity.	White, round elevated centers and spreading hairs.	Seashell-pink, rope-like, with coremia.	White, tallow-like; few coremia.
C. rubrobrunneum	Orange-pink, flat, corrugated; submerged cephalosporia.	White, edges submerged, later cottony.	Coral-pink, corrugated; few cephalosporia.	Deep pink, flat, moist; cephalosporia.	Pale pink, wrinkled, much submerged, sterile.	Pale orange, flat, moist; few cephalosporia.	White ring, slight turbidity.	White-silvery, hard cerebriform colony.	Frosted seashell-pink, cerebriform; many cephalosporia.	White, tallowy.
C. Serrae	Gull-gray, short-fibrous; submerged chlamydospores and cephalosporia.	White, heaped, rough, edges submerged.	Steel-gray; many chlamydospores; few cephalosporia.	Silvery-gray, powdery; much submerged chlamydospores; few cephalosporia.	Gray-powdery, flat, wrinkled; chlamydospores.		Downy white pellicle.	Yellow, powdery tops, wrinkled.	Pale pink, cottony, slightly wrinkled.	White, tallowy, some fuzz; some coremia.
C. Stuehmeri	White, short, rough surface, submerged; many cephalosporia.	White, flat, granular, fimbriate.	Pale pink; nodular coremia; many cephalosporia.	Pale pink, slightly elevated, dry; many cephalosporia.	Opaque, round elevated, radial furrows, sterile.	White, moist; some coremia; few cephalosporia.	White yeasty ring, turbidity.	White, hard, wrinkled shell, orange below; coremia.	Pink, wrinkled below; coremia.	White, tallowy.
C. symbioticum		Pale pink, mostly submerged; few coremia; many cephalosporia.	White, matted with pink coremia, medium grass-green.	Chlamydospores; submerged cephalosporia.	Pink, flat, wrinkled; submerged cephalosporia and chlamydospores.		Yellow-green pellicle; pink submerged hyphae.	Pink then black, mostly submerged; cephalosporia and chlamydospores.	Orange coremia and pink-yeasty then black; chlamydospores.	White powdery.
Clonostachys Araucaria	Citron, in medium too, powdery, submerged; few aerial cephalosporia.	White, slightly raised, granular center, orange below; cephalosporia.	Pink, cottony; many chlamydospores; few cephalosporia.	Pale pink, scattered, small dry heaps; cephalosporia; chlamydospores.	Cephalosporia.	Pale pink, dry, powdery; many cephalosporia.	Powdery white pellicle, some hyaline basal growth.	Pale pink, cottony; cephalosporia.	Shrimp-pink, downy, umbonate, wrinkled below.	Pinkish-white, short-haired fuzz.
Clonostachys Araucaria var. *rosea*	Primuline-yellow, in medium too, powdery, submerged; few aerial cephalosporia.	White-opaque, discrete tufted colonies; few cephalosporia.	Chalky white, heavy, flat-matted growth, sterile.	Opaque, flat; cephalosporia.	Chalky-white, matted, coarse; chlamydospores; cephalosporia.		White yeasty ring, slight turbidity.	White, yellow below, wrinkled.	Pink center, rough, hairy, wrinkled below; cephalosporia.	White, small cottony, sparse growth.
C. Araucaria var. *confusa*	Orange-pink, powdery; cephalosporia.	Pink, raised, fuzzy.	Pale pink, flat with powdery tufts; cephalosporia.	Pink, scattered tufts, dry; cephalosporia.	White, rough, matted; cephalosporia.	Pale pink and white, downy.	Pink-white, powdery pellicle, liquid clear.	White, matted, yellow below, wrinkled.	Pink-orange, thick-matted, wrinkled below; few cephalosporia, swollen thin-walled cells.	Pink, fuzzy, good growth.
Gliocladium deliquescens	White, green dots, mostly submerged scattered threads.	White, fuzzy; submerged cephalosporia.	Blackish-green, medium brown; aerial cephalosporia.	Green dots scattered, sparse growth; loose spores.	Sterile.	White; many cephalosporia.	Gray-green surface pellicle, hyaline below, liquid clear, large cells; no cephalosporia.		White cottony center; cephalosporia at edge.	Delicate green fuzzy growth.
G. fimbriatum	Dark green, medium yellow-brown, felted; cephalosporia and akinetes.	Dark green, white edges; cephalosporia.	Deep green, medium tan, matted, moist; many cephalosporia.	Green, moist; many cephalosporia.	Yellow-green, powdery, moist; cephalosporia.	White, medium yellow-green, cottony.	Pale green, feathery pellicle, hyphae in liquid too.		Meadow-green, edges white, rapid flocculent growth, medium olive-yellow; many cephalosporia.	White hairy growth.
G. Vermoeseni	Pink powdery; many cephalosporia.	White, sparse mycelia; pink cephalosporia.	White, fibrous; chlamydospores; submerged cephalosporia.	Pink, powdery, elevated; few small cephalosporia.	White, powdery, sterile.	Transparent, scattered.	Pink ring, liquid clear.	Pink spore balls, white cottony hyphae.	White mycelia; lavender spores; rapid growth.	No growth.

may be drawn here, indicating as favorable media the glycerine, Sabouraud's dextrose, and Czapek's agars; and as unfavorable, nitrate and Gorodkova-Maneval agars; as less so, corn meal and potato-dextrose agars, carrot slants, and liquid media. Growth on the first group is typified by abundant large, aerial mycelia with comparatively few types or numbers of spore forms, deeper internal coloring with little if any exuded pigment, much guttational water, and a convoluted colony. Unfavorable conditions are recognized in sparse surface growth of long slender filaments, a tendency to submerged mycelium, relative increase in reproductive bodies such as chlamydospores, conidia, and coremia, dry powdery growth, paler or tending toward yellow rather than orange pigmentation endogenously, with frequent exogenous coloration of the medium.

In the next section some of the prominent factors and theories for cultural deviations which seem especially applicable in the case at hand will be more fully discussed.

B. FACTORS INFLUENCING MORPHOLOGY AND PHYSIOLOGY

Of the numerous variables possibly relevant, the present discussion is limited to hydrogen-ion concentration, surface and oxygen tensions, and kinds of nutrients. Temperature, doubtless influential, will not be considered, since all were grown at 30° C.

1. Hydrogen-ion concentration.—It must be remembered that the hydrogen-ion concentration refers to the relative acidity or percentage of dissociation as contrasted with the total acidity; although the latter factor, as well as the chemical nature of the acid itself, also influences growth. Moreover, microorganisms contain natural buffers and have to a limited extent the power to adjust the pH of a medium toward the optimum as a result of their activity.

The media utilized covered a wide natural range of H-ion concentration, from Raulin's (pH 4.15) to Endo's (pH 7.5), with growth upon all. An artificial series from pH 3.8 to 7.6, maintained upon glycerine agar (table II), gave the most striking results in the tendency towards submerged growth at the

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higher concentrations of pH. At pH 4.4 and below and at pH 7.6 the media did not solidify. It is interesting that *Gliocladium deliquescens* grew in the liquid at the higher acidities, but formed a pellicle at the higher alkalinities. On the other hand, *Torulopsis mucilaginosa* grew extensively and normally upon wort agar (pH 4.8) but with mainly submerged cephalosporia upon nitrate agar (pH 5.5); whereas the true molds showed an inundated state upon the latter and abnormal aerial conidiophores even upon wort.

Organisms listed as *Monilia albicans* have been extensively used in physiological studies. Marantonio ('93) stated that at the higher acidities there was a greater quantity of mycelium; and that there was a tendency to filamentous growth on liquid, with sprouting almost exclusively upon solid media, or hyphae only on old cultures. According to Fineman ('21), mycelial growth was better under low surface and oxygen tensions, but the yeast form predominated upon solid media, simple carbohydrates, and a low acidity. Mallinkrodt-Haupt ('32) stated that the yeast form predominated in a strongly acid medium, whereas the filamentous occurred in a very alkaline one; and he also mentioned that H-ion concentration influenced the metabolism, enzyme actions, morphology, toxin production, and immunological characteristics.

Buchanan and Fulmer ('28) found that some of the molds could tolerate a range of pH from 1.6 to 11.2, but that there was an optimal section with atypical growth deviations on either side. With fermenting yeasts alcohol was formed under acid conditions, and glycerol as an intermediary under alkaline ones.

In *Endomyces capsulatus,* Moore ('33) said that in acid media the tendency was towards abundant budding, numerous conidia, and longer narrower cells; contrarily, on alkaline substrata there was an inclination towards shorter thicker cells with yeast-like formations and fewer conidia.

Hewitt ('33) said that the pH had an effect upon the oxidation reduction system, which resulted in an alteration of the ionic equilibria. Clark ('22) thought that the H-ion concentration functioned chiefly as a conditioning agent and was only in-

TABLE II

EFFECT OF HYDROGEN-ION CONCENTRATION—GLYCERINE AGAR

Organism	pH 3.8	pH 4.4	pH 5.0	pH 6.0	pH 6.4	pH 7.0	pH 7.6
Allescheria Boydii	No growth.	No growth.	Gray, velvety, somewhat submerged, wrinkled.	Very slight growth.	Gray-velvety growth.	Luxuriant gray down, black center, moist.	No growth.
Clonostachys Araucaria	No growth.	No growth.	Fine mycelium, pink, powdery; one coremium.	Pink, fluffy, powdery.	Pink and white down, wrinkled, moist.	White down becoming pink, powdery, dry.	No growth.
C. Araucaria var. *confusa*	Slight growth.	No growth.	Pink-orange, powdery; few coremia.	Pink, cottony, quite coarse.	Very cottony and pink, moist.	White then salmon, downy.	Small amount of submerged growth.
Cephalosporium Serrae	No growth.	No growth.	Pink, coarse growth, soon becoming black with chlamydospores.	Heaped compact colonies.	White, wrinkled, cottony; coremia.	White, tufted.	No growth.
Gliocladium deliquescens	Submerged growth; cells thin-walled, granular, short, contorted, with intercalary ones of swollen walls; no cephalosporia.	Colorless growth in liquid; cells less contorted and longer, granular; some cephalosporia.	Mostly submerged growth, white with green dots.	Fine white surface growth with green slime balls, akinetes; cells long and narrow, heterogenous contents.	Flocculent white growth with green slime.	Flat dull surface growth and somewhat submerged.	Heavy pellicle and radiating hyaline hairs; cell walls thick, hyaline, contents granular; few terminal chlamydospores; no cephalosporia.

directly concerned with chemical transformations closely related to life processes.

2. Surface tension.—Surface tension is the cohesional pull of the internal and adjacent molecules upon those of the surface layer. It is particularly important in the life of the organism in relation to adsorption and absorption. This tension produces surface energy, which, unhindered, effects the assumption of minimal surface for a given volume, i. e., a sphere. At the interfaces which are sites of chemical and physical activity, surface tension is constantly being opposed. Anything causing a lowering of surface tension will permit a larger surface; for example, mycelial growth will be favored, but under the other condition the cell size will be limited and yeast-like proliferation result. The appearance of filamentous forms in liquids is probably a reaction due partly to surface tension, and represents unfavorable adaptation to an unfamiliar substratum.

Artificial reduction of surface tension has been secured by Fineman ('21) with the addition of sodium ricinoleate to a medium with dextrin solutions, but not those containing galactose. Glycerine has also been found to lower surface tension and increase mycelial growth. Upon the basis of similar observations, Czapek ('11) was led to stipulate the necessity of lipoids in the surface membrane. Surface tension is greatly affected by the polar orientation of interfacial substances, the amount of ionization, and the pH (which determines the basic or acidic activity of amphoteric proteins).

Larson ('21) attributed his success in growing normal pellicle-forming bacteria on the bottom and vice versa by lowering or raising surface tension, to the non-wetting properties of the lipoids in the surface pellicle. Buchanan and Fulmer ('28) reported similar results in making *Clostridium tetani* grow superficially, but they suggested that the surface-active solutes concentrated in the surface boundary might also cut down the solubility of oxygen. They also mentioned the tendency of *Bacillus subtilis* to sporulate in media of low surface tension. Oxygen tension, availability of nutrients, etc., may overbalance or at least modify the effects of surface tension.

3. Oxygen relationships.—The aerobic and anaerobic states are dependent upon the oxygen relations. Topley and Wilson ('29) stated that anaerobes were not supersensitive to oxygen itself, i. e., that oxygen was not toxic, but that hydrogen peroxide was produced abundantly in the presence of free oxygen for whose destruction no catalase or equivalent system was present. This theory also explains why anaerobes can sometimes be cultivated with an aerobic form in air, the latter destroying the peroxide.

Fineman ('21) found mycelium formed in *Monilia albicans* on surface agar slants cultured in carbon dioxide. The usual correlation of budding and high oxygen tension and of mycelium and low oxygen tension was noted, but there were exceptions. According to Fuchs ('26), reduced oxygen tension favored sporulation in yeasts. Dodge ('35) stated that lowered oxygen tension favored hyphal production to some extent.

The metabolic relation to oxidation is also intimately associated with the substratum. Stephenson (Buchanan and Fulmer, '28) found the normally aerobic *Bacterium coli (Escherichia coli)* developing anaerobically during the first stages of glucose breakdown, and thereafter aerobically. However, if grown in pure oxygen it utilized such from the beginning, nor was there any increase in the ratios of glucose decomposition, but the active metabolism persisted longer than in the former case and the acidity increased more slowly. Thus the apparent effect of the increased oxygen was to induce further breakdown of some acid product of the fermentation of glucose. An increased oxygen tension accelerated the respirational rate when dextrose was replaced by ammonium-lactate, and made possible the derivation of carbon from salts of acetic or succinic acids but under aerobic conditions only. In a synthetic medium of simple organic acids (such as lactic, succinic, fumaric, or pyruvic) to which nitrate was added, the organism could grow anaerobically by utilizing the reduced nitrate as a hydrogen acceptor or oxygen donator. Buchanan and Fulmer ('28) further noted that in *Bacterium coli (Escherichia coli)* and *Clostridium Welchii* smaller amounts of carbon dioxide were produced aerobically than anaerobically.

Hewitt ('33) offered an explanation of aerobisis and anaerobisis on the basis of oxygen-reduction potentials in place of the view that anaerobes have no catalase. He showed that aerobes (such as staphylococci, streptococci and pneumococci) reduced the broth medium whereon they were cultivated, which had an initial potential of almost –0.3 volts, to as low as –0.15 to –0.20 volts. Anaerobes, on the other hand, could not begin growth on such a medium, but with pieces of meat in the broth the potential became reduced to –0.2 volts, due to a reversible oxidation-reduction system therein. Such potential was not changed by staphylococci which had reached their minimum, but was lowered by anaerobes to a limit of –0.4 volts. The preliminary reduction of the medium might be effected artificially by four methods: (1) hydrogen in an anaerobic jar, (2) pure nitrogen, (3) various chemical-reducing agents, (4) growth of aerobic organisms.

4. Kinds of nutrients.—Besides the above-mentioned interrelations of oxidation and nutrient necessities, there have been some definite observations regarding the nature of the latter alone.

Talice ('30), in his extensive study of media and conditions favoring the formation of hyphae, found that mycelium was formed the first few days and then mainly yeasts, with the filamentous form only in contact with the agar surface. Surface tension may enter here and oxygen phenomena, since the inside of a colony is under reduced oxygen pressure. He noted that normal yeast forms produced hyphae with dextrin peptone media or glucose, and to a lesser extent with protein. Incidentally, he regarded the yeast state as a senescent one.

Plaut ('13) found yeasts in sugar-rich media and mycelia in sugar-free nitrogenous substrata. Buchanan and Fulmer ('28) spoke of the "protein-sparing" effect of dextrose. Topley and Wilson ('29) thought that there was no such effect, but rather an inhibition of bacterial growth, due to the rapid increase in hydrogen-ion concentration of those media containing a fermentable carbohydrate.

Further conclusions have been drawn from work with *Monilia albicans.* Roux and Linossier ('90) stated that the complexity of morphological structure increased with the molecular weight of the food elements, i. e., yeasts in simple sugars, and filaments in complex carbohydrates. Such statements have not been fully confirmed. According to Mallinkrodt-Haupt ('32), the yeast form appeared in the tissues of the host, but in culture the mycelial one. Discordantly, Fineman ('21) reported both conidia (yeasts?) and mycelium in the host, but only the one or the other in culture. No explanation was offered but if such be true, the heterogenous nature of the internal environment may be responsible.

5. Discussion.—It soon becomes obvious, when trying to check physiological causes and effects, that there are many variables concerned, making it practically impossible to attribute a definite reaction to one alone. Nevertheless, some likely influences can be designated.

There is an optimum range of hydrogen-ion concentration between pH 5 and pH 7, and the best is around pH 6.4 for the majority of these forms. Below and above the range the growth is restricted and the relative number of reproductive bodies, especially chlamydospores, augmented. Moreover, the maladjustment is exemplified by atypical colony characters—i. e., submerged contorted growth at the higher acidities and aerial if scanty growth with increased alkalinities.

The forms studied show strong aerobiosis, tending to form pellicles on liquids, but are occasionally submerged with modified hyphal growth due to other more influential causes. If other conditions are unfavorable, however, all of the organisms may grow immersed even on a solid substratum. These molds seem to require media rich in both carbohydrates and proteins. The organic nitrogen foods are more easily assimilated than the mineral salts; and sugars better than starches. Glycerine is readily utilized.

Some of the present results may be explained as follows: the relative acidity may occasion the submerged growth in Czapek's medium; low carbohydrate supply in nitrate and Gorodkova-Maneval agar necessitates the utilization in the first case of nitrogen of a mineral nature and in the other of an organic nature, with comparable results; utilization of complex plant carbohydrates *in situ* in corn meal, potato, and carrot, with increased production especially of coremia; strong aerobic tendencies evidenced by heavy pellicle, with the species of *Cephalosporium* best able to grow anaerobically if atypically submerged.

Although some have regarded the yeast type as a senescent primitive form (Talice, '30), present evidence would seem rather to indicate a reduced type. The yeast has become a highly specialized saprophyte, i. e., adapted to growth in sugar solutions. Such a habitat has effected extreme morphological changes which caused sexual degeneration, and the almost exclusive assumption of the budding state due probably to chemical and physical causes associated especially with surface tension and the nature of the substratum. Perhaps the status of oxidation-reduction potential in the sugar medium may elucidate upon the morphological effect. Hewitt ('33) lists carbohydrates as having an unknown reversible oxidation-reduction system.

The insertion of the yeast stage normally in a filamentous form is interesting too. For example, the smut sporidia propagate rather indefinitely by budding. Conidia of the Plectascales have also been found upon occasions to bud for various time intervals. Fuchs ('26) gave a review of the early concepts which regarded the sprouting interval as a part of the life cycle of filamentous forms. He found that *Aspergillus Oryzae,* especially on wort and wort gelatine, remained permanently in the yeast stage. Such data have not been duplicated to the knowledge of the author. The latter media have been found unfavorable to the molds, probably because of the large amount of sugar, reduced proteins, and also relatively high acidity. In pseudomycelial forms—*Monilia* in the general sense of the name—the yeast state is prevalent under favorable conditions, and the mycelial one under unfavorable situations according to Henrici ('30).

C. COLOR

The two dominant colors found in this group are green and red in various shades, although one organism was gray and three were sporadically yellow. The first pigment seems quite constant under all conditions and is apparently in the mycelial protoplasm as well as the spores. In *Gliocladium deliquescens* optimal growth shows almost complete covering with a green slime whose color is doubtless due to the numerous spores included. *Gliocladium penicilloides* generally is chalky-white, but sometimes becomes pale yellow and on glycerine agar exudes the same pigment into the medium. A similar condition is present in *Clonostachys Araucaria* and its variety *rosea* on nitrate medium. The pigment is water soluble.

It is the various red and orange shades which attract particular attention. The pink pigment of *Gliocladium Vermoeseni* is obviously contained within the spores since the mycelium is a white flocculent growth. In the species of *Cephalosporium* the pigment is dispersed within the mycelium, but its intensity or mere presence is conditioned by varying factors of light, temperature, substratum, oxygen relations, associated probably with general metabolism. A few observations are to be mentioned here, although little experimental work was done upon their nature.

Cephalosporium kiliense when growing most luxuriantly in the light is a rich apricot-orange; but as the culture stales, or on media low in accessible nutrients, or in the dark, it fades to a pale yellow-orange or even almost white. *C. niveolanosum* is a paler pink at best and is white if kept in the dark (at the same temperature) for even a few days. The color of the *Torulopsis mucilaginosa* is more persistent, but in one case when a culture was placed in the refrigerator (13° C.) for two months it became white and remained so on all subsequent transfers.

An attempt was made to extract the pigment of *Cephalosporium kiliense* with various solvents. With concentrated sulphuric acid the specimen became blue-green immediately, and the liquid later had a cherry-wine color due perhaps to the dissolution of agar. Concentrated hydrochloric acid turned the solution a blue-green color which faded to yellow. Fuming nitric acid, as well as 20 per cent sodium hydroxide, dissolved the fungus and medium with no color reactions. There was slight solubility in chloroform as well as in hot 95 per cent alcohol and in lactic acid. Entirely ineffective were petroleum ether, benzol, oxalic acid, salicylic acid, acetone, formaldehyde, and ethyl-ether. The pigment was especially soluble in acetic acid and upon evaporation yielded a yellow-orange mass which appeared amorphous under the microscope. This observation is insufficient to identify the pigment, although it seems related to xanthophyll. The reaction with hydrosulphuric acid was not the typical one of lipocyanin.

Color variations have been occasionally noted in fungi, and Zopf ('90) worked out some rather complicated methods of extraction and determination. Palmer ('22) stated that in some cases the reds and yellows were doubtless due to carotinoids, whereas in other cases they were not, as in chrysophanic acid. Pigments have also been cursorily noted in the bacteria, and it has been possible to separate rather constant strains, and at other times conspicuous color variants have been noted in a colorless colony or vice versa. Little or nothing is known of the chemistry or physiological purpose of pigments. Some theories have been promulgated: reserve of oily nature in the rust spores (Zopf, '90), a protective function to cellular enzymes (Went, '04), and an oxidation pigment. It may be that the pigmentation is largely associated with conditions of acidity and alkalinity in the medium, as Thom ('26) has suggested, yellow occurring in acid conditions and red in alkaline.

In the cases at hand—*Cephalosporium niveolanosum* and *C. kiliense*—it is possible that the pigment is a reserve and indicates excessive anabolism, but chemical investigation would be necessary to substantiate the view. A protective function seems quite logical whether for enzymes or the cytoplasm itself. The association with respiration seems unnecessary, since existence is possible without pigment in these species and in closely allied ones (e. g., *C. rubrobrunneum* and *C. Stuehmeri,* separated almost entirely upon the presence or absence of pigment). At any rate the problem is still an open one and possibly full of significance.

According to Henrici ('30), the pigment production in *Actinomyces* was more constant and also more striking in media of rather low nutrient value, which is the reverse of the present findings except for *Clonostachys Araucaria,* where abnormal pigments are released in the medium. Colley ('31) enumerated changes in color of *Serratia marcescens* and *Azotobacter chroococcum* upon the addition of certain salts to Bacto-agar. In the latter case the color was supposedly due to the oxidation of tyrosin to melanin, the tyrosin being formed in the breaking down of proteins when the carbohydrate supply was insufficient and unsuitable; but on the addition of zinc salts the color production was due probably not to the exhaustion of carbohydrate but to unfavorable conditions preventing its use.

The intense yellow water-soluble pigments diffusing into the medium is obviously a metabolic product, perhaps the chrysophanic acid of Zopf. In the case of the nitrate medium, at least, it might indicate unfavorable growth, although it doesn't especially appear so for all media, certainly not with glycerine agar.

D. POLARIZED LIGHT

Since, as has been stated, the walls of reproductive cells are sometimes of different constitution from vegetative cells and since they might possibly have peculiar physical characteristics, they were studied with a polarizing microscope. Agar-slide cultures and hanging drops were employed under high power and oil immersion. No structural differences were detectible by this method, although the twisted nature of the wall in the phialide was most conspicuously brought out. Sometimes, in ordinary mycelia, adjacent cells were noted to refract differently. This is presumably due to the different orientation of the cells caused by twisting, but it may have been that some of them were beginning to form chlamydospores and hence were of different structure.

V. Fungi Imperfecti and Sexualty

Lindau's ('07–'10) treatment of the Hyphomycetes has been followed. The subfamily Cephalosporieae is separated from the Aspergilleae in that the spores are in balls in the former and in chains in the latter. The Aspergilleae include *Penicillium* and *Gliocladium. Clonostachys* belongs in the Verticillieae. *Allescheria Boydii,* also described in different developmental stages as *Cephalosporium Boydii* and *Dendrostilbella Boydii,* because of its perithecial fructification, is placed in the Eurotiaceae of the Perisporiales. Clements and Shear ('31) have made *Allescheria* a synonym of *Eurotium.* The fertile state of *Gliocladium penicilloides* placed it in *Eurotium insigne* according to Winter ('87), but this disposition was later disputed by Matruchot ('95). Thom and some later workers have segregated the perfect forms of *Aspergillus,* as well as imperfect forms with similar conidia, in the Aspergillaceae, close to the Gymnoascaceae of the Plectascales.

A. EXPERIMENTS TO INDUCE SEXUAL FRUCTIFICATIONS

The author at first believed that the imperfect stages were merely labile and that perfect fructifications might possibly be produced by various special methods. In no case were these successful. After elaborate experimentation with a number of algae and other forms, Czurda ('33) came to the conclusion that copulation was caused only by pH and internal disposition, and since the latter was intangible, pH was the only factor subject to investigation. The present experiments did not indicate that there was a specific pH, at least one which was solely responsible. The usual media were employed to evoke fertile stages. Drying or maintaining at low temperatures merely showed the extreme longevity and resistance of these organisms. It was thought that by crossing nearly related species and varieties fertile stages might be attained. Therefore cross-inoculations were made of *Clonostachys Araucaria* and its varieties *rosea* and *confusa,* as well as of all of the species of *Cephalosporium.* The only noticeable result was an aversion of *Clonostachys Araucaria* and the variety *confusa.* The rest intermingled promiscously. Agar micro cultures, as well as Petri plate specimens, were observed, and although no particular hyphal fusions were noted, neither were there ordinarily signs of repulsion.

B. INTERPRETATIONS REGARDING LOSS OF SEXUALITY

Ramsbottom ('33) believed that heterothallism and saltation were responsible for the origin of the Hyphomycetes.

> Thus theoretically we may regard one of the Fungi Imperfecti as able to carry on the influence of the environment. If this is so it is possible that a permanent change may be brought about. . . . If such changes occur naturally then we may get differences which might throw heterothallism out of gear.

It was his suggestion that these should not be regarded as species but rather as form-species. Hansen and Smith ('32) held similar views on *Botrytis cinerea* which theoretically has a perfect stage in *Sclerotinia Fuckeliana.* They discussed previous explanations as (a) impure material, i. e., a complex of established types on the host which must be separated, (b) mutation, (c) hybridization, (d) microconidia—some possible significance but little evidence that they even have enough power of germination to play an important rôle, (e) anastomoses, hyphal fusions, heterocaryosis, i. e., condition of nucleus containing two or more genetically different nuclei, (f) mixochimaera (no cytological evidence of this yet). Their cytological studies showed that the mycelial cells and conidia were multinucleate. Further analyses of types were made by 128 single spore cultures, from which were selected 8 showing the greatest morphological variation. Twenty-five single spore cultures were made of these and a record kept for a number of generations. Hansen and Smith concluded that the regularity and completeness with which the homotypes separated from the heterotypes indicated that the character-determining elements were discrete units of limited number, suggestive that the basic unit of the individual was the nucleus and not the cell. Therefore a multinucleate spore, a colony really, cannot give rise to a genetically pure culture unless all of its nuclei are genetically identical. Gregory ('35) suggested the following explanation for loss of sexuality in the dermatophytes, namely, environmental factors, elimination of sexuality (negative adaptation to parasitism), and heterothallism.

Although the species here studied are uninucleate, heterothallism may well play a part. It is not unlikely that there are many races, new ones constantly being formed by the mechanism of hyphal anastomoses, and some of these are actually antagonistic although morphologically very similar. Perhaps some compatible strains have died out, leaving imperfect halves to vegetate ceaselessly unless by saltations new harmonious ones shall arise.

VI. Medical Aspects

A. PATHOGENICITY

Of the forms isolated from skin lesions—*Allescheria Boydii* and the species of *Cephalosporium*—there is some doubt as to real pathogenicity. Of the *Cephalosporium* species treated by Dodge ('35), only *C. Serrae,* of keratomycosis of the eye, and *C. Doukourei,* of a gummatous lesion, are said to be pathogenic to laboratory animals.

Allescheria Boydii, isolated by Boyd and reported by Shear from a case of granular mycetoma of man, was thought by them to be a contaminant and not an anaerobic form because of its peculiar morphology within the foot and its inability to infect laboratory animals. A number of other Hyphomycetes, including species of *Indiella, Glenospora, Scedosporium, Aspergillus,* and *Penicillium,* have been reported as etiological agents of maduromycosis. At present it would seem that such were purely accidental contaminations, which may be fatal but are not naturally so.

The author secured species of *Penicillium* and *Aspergillus* frequently from eczematoid infections at the Barnard Free Skin and Cancer Hospital, and the *Cephalosporium symbioticum,* with the intimately associated *Torulopsis mucilaginosa,* from a particularly severe case of dermatomycosis. She also isolated the latter with a similar co-form which was eliminated in subsequent dilution cultures from a case of pemphigus vulgaris. Apparently the same red yeast was found by Engelhardt ('27) in a case of pemphigus. He illustrated the organism as it grew in the host, i. e., filamentously, similar to the condition here found on nitrate agar, although no cephalosporia were shown. Whether such organisms are purely saprophytic secondary or primary invaders, or necessary symbionts of forms better believed to be pathogenic (Hartmann, '26), in the present case of a *Cephalosporium* and *Torulopsis mucilaginosa,* is as yet unknown; but immediate evidence at least indicates that they have rather specialized habitats, and constitute a group which should be investigated from a pathogenic viewpoint.

The various species of *Cephalosporium* have been regarded as accessory perhaps symbiotic saprophytes. Hartmann ('26) found *C. niveolanosum* associated with *Trichophyton gypseum* and left the question open. So great a number have been secured from superficial lesions that although animal inoculations may be negative (perhaps due to methods of inoculation) a parasitic nature is certainly suggested. The author has found species of *Cephalosporium* from superficial lesions of the glabrous skin especially. Various species of *Penicillium* and *Aspergillus* are even more common. *Alternaria, Sarcina lutea,* and *Torulopsis mucilaginosa* are likewise frequently found. Some species of *Cephalosporium* are also reported as saprophytic in nature on soil or humus. None of these were studied; but an organism treated by Buchanan ('11) as *Cephalosporium Pammelii* appears different from the skin group, at least in the possession of multiseptate conidia, whereas only uniseptate ones are found in the group here investigated. Species of *Cephalosporium* have also been found on insects (Petch, '28) and as causative agents in various plant diseases. In the Dutch elm disease it is only a stage of an organism, of which the perfect stage is *Ceratostomella Ulmi* of the Sphaeriales. Reddy and Holbert ('24) gave a good description of the effects of *C. Acremonium* Corda emended Fresenius, upon corn, causing the black bundle disease. In all these cases it is the transporting system which is attacked. The habitat would certainly indicate parasitic tendencies which might as well be developed in animal tissues as in those of the plant.

B. OCCURRENCE OF GRANULES IN CULTURE

One strange cultural condition certainly deserves further mention. On blood serum *C. rubrobrunneum,* after several weeks, produced numerous small white spherical bodies several mm. in diameter. Under low power they appeared surrounded by a fine felt of white to pale pink mycelium (pl. 6, figs. 15–16). These proved to be very hard and when crushed revealed a white chalky interior. Under higher power numerous linear crystals radially arranged were seen. These crystals did not stain with lacto-phenol and cotton blue, nor with glycerine-eosin, and there was no definite cellular mass visible, although there were some irregularly shaped stainable masses. The latter were suggestive of the granular bodies occurring sometimes in the animal body, in fact quite definitely characteristic of certain diseases. Of late such have been noted more frequently upon artificial culture (Area Leão and Lobo, '34).

Two recent papers (Almeida, '34, and Weidman, '32) gave rather fully the history of granular occurrences. These varied in the host from the capsules of bacteria, *Saccharomyces hominis* and *Cryptococcus histolyticus,* to the radiating structures of purely membranal or cellular nature, as in *Aspergillus,* and the definite extra-cellular grains produced in *Actinomyces,* Madura foot, and the like. They have been interpreted as a reaction to the host comparable to distortions of haustoria, as living structures capable of absorbing food, etc., or as mere dead extensions of the membrane. They are compared to the "Hülle" cells first mentioned by Eidam ('83) in species of *Aspergillus*—all strains of the *A. nidulans* group and some strains of *A. terreus, A. flavipes,* and *A. ustus.* These cells develop from older mycelia as smaller branches, and the terminal and subterminal cells become surrounded by a very thick mucilaginous wall, comparable to that of *Saccharomyces hominis* produced in the host. The other structures have been evoked upon organic media frequently—a fact which eliminates a vital cause and probably puts such formations on a chemical basis. Weidman ('32) compared these incrustations to the similar mechanism in the "Dauercyst" of protozoa, i. e., that the capsule (especially) is protective against adversity and preparatory to reproduction, as likewise the asci in fungi are reproductive cells whose development is stimulated by unfavorable conditions. He does soften this rather far-fetched homology of reproduction and adverse conditions (although such was probably the primitive state) by saying that possibly reproduction could be absolutely separated from environmental factors. He thought that adverse conditions might also be assumed for fungi in the comparatively anaerobic and warm environment of the mammalian tissue.

Incrustations of the type found by the author are extramatrical, of the "calcareous incrusting substance" type in Weidman's classification. Their origin and purpose are poorly comprehended, but they are probably reminiscent of pathological conditions in the host. Various solvents (concentrated acids of sulphuric, nitric, and hydrochloric) were tried on these crystalline masses which it was conjectured might be calcium oxalate. The most rapid action occurred in hydrochloric acid, releasing numerous square-oblong flat crystals and some loose cubical arrangements of about sixteen small ones; and the remainder of the ball appeared segmented with fine long crystals which protruded radially. In nitric acid there was much bubbling, and the balls assumed a rougher appearance under the microscope but dissolution was very slow. The bubbles were colorless, of a peculiar hexagonal outline, and their heavy part contained small crystalline fragments. In sulphuric acid there were crystals similar to those in the hydrochloric acid, and some hollow ones. It could not be noted if the ball were actually diminishing in mass, but the liquid around it seemed of a deeper yellow than the normal acid, perhaps indicating solution.

VII. Taxonomy and Morphology

The general classification of this group has been given in a previous section, and only the individual members will be discussed here. Due to the temporary nature of this group, much changing of nomenclature is both unnecessary and confusing. A couple of very obvious errors are corrected, however, i. e., in regard to the former *Gliocladium roseum* and *Clonostachys spectabilis.* The new organism isolated by the author is described as *Cephalosporium symbioticum.* Descriptions are based mainly upon giant colonies grown upon glycerine agar. The names of the colors are according to Ridgway ('12).

Allescheria Sacc. & Syd. in Sacc. Syll. Fung. **14**: 464. 1899.

It was thought that if this form were included with a known fertile stage more light might be shed upon the significance of the conidiophore. Unfortunately, the entire life cycle was not recalled and a complete cytological study was impossible. The conidiophores are poorly developed and resemble somewhat those of *Cephalosporium;* but the dimorphism of conidia is a constant feature, and the soft downy colony is quite distinct from the smooth rope-like one of *Cephalosporium;* so that it is doubtful if even the spore-ball stage should be included in that genus. Type: *Eurotiopsis Gayoni* Costantin.

1. ALLESCHERIA BOYDII Shear apud Boyd and Crutchfield, Am. Jour. Trop. Med. **1**: 258–268. 1921; Mycologia **14**: 239–243. 1922. Pl. 1, figs. 18–28.

Colony: pale gray velvety, of short filaments, sometimes becoming greenish in spots, or with much longer pile and approaching white, cerebriform and growing into medium at edges; reverse slightly hardened and wrinkled; growth relatively slow.

Conidiophores: simple; phialides on aerial mycelia, 3–20 μ long, septate or not, cutting off oblong conidia which adhere irregularly; spores with two oil droplets, ovoid, capable of immediate germination, 8 × 5.5 μ, heavy-walled spores occasionally preceding hyaline ones, the former characteristic of single-spored phialides of coremia, 15.5 × 4 μ; terminal chlamydospores 8.5 × 11 μ.

Habitat: isolated from a case of granular mycetoma in a negro. Culture of Shear, from Baarn.

CEPHALOSPORIUM Corda, Icon. Fung. **3**: 11. 1839.

Colony: smooth, much corrugated, rope-like, forming a consistent layer, slightly adherent.

Conidiophores: simple or with few short lateral branches along cords of hyphae; hyphae repent, phialides long and slender, only slightly narrower at tips than at base, bearing spores in slime balls; coremia frequent on certain media; chlamydospores present. Type: *C. Acremonium* Corda.

The six species here studied form quite a homogenous group. *Cephalosporium symbioticum* and *C. Serrae* vary most widely, the former including yeast-like growth and becoming fuscous with chlamydospores, the latter becoming pubescent and also dark with chlamydospores.

KEY TO SPECIES OF CEPHALOSPORIUM

1. Producing some yeast-like growth on solid culture, apricot-buff, becoming fuscous .... *C. symbioticum*
1. Not found to produce yeast-like growth in culture 2
2. Colony white with a rough dry surface, eventually becoming fuscous due to chlamydospores .... *C. Serrae*
2. Colony not becoming fuscous 3
3. Colony moist, rope-like, turbinate 4
3. Colony dry, often with a bloom, irregularly dissected 5
4. Colony ivory to pale pink; conidiophores simple; spores 6 × 3 μ. . *C. niveolanosum*
4. Colony soon becoming deep orange; conidiophores simple or branched; spores 2–4 × 1.5 μ .... *C. kiliense*
5. Surface of colony smooth, cerebriform, seashell pink; spores 7 × 1.5 μ .... *C. rubrobrunneum*
5. Surface of colony short-pubescent, slightly convolute, white to salmon-buff; spores 4–5.5 × 2.5–3 μ .... *C. Stuehmeri*

1. CEPHALOSPORIUM RUBROBRUNNEUM Nannizzi, Tratt. Micopat. Umana [Pollacci] **4**: 455. 1934. Pl. 2, figs. 22–25.

C. rubrobrunneum cerebriforme Hartmanni Benedek, Arch. f. Derm. & Syphilis **154**: 166. 1928.

Colony: at first an irregular small dry pink heap in center, spreading irregularly for about a cm., then flat, about 1 mm. thick, moderately dry, whitish pubescent at edges, center of coremia becoming frosted, seashell pink, very cerebriform, edges abrupt; little guttational water; reverse corrugated and consistent, pale orange, little color diffusing into medium; no coremial balls.

Conidiophores: borne on ropes of numerous hyphae up to 11 μ in diam., surrounded by thick mucus, spreading from both sides, non-septate, up to 30 × 2–3 μ, producing spores in balls 5–15 μ in diam., or irregularly clinging along sides of phialides in packets; spores oblong-ellipsoid, 7 × 1.5 μ, or almost spherical, 2–2.5 μ, sometimes slipping past each other at only a slight angle and forming a column; terminal chlamydospores on wort agar, 17 × 14 μ, globular crystalline bodies on serum (see pl. 6, fig. 9, and discussion p. 35).

Habitat: isolated from a case of dermatitis in Frankfort, a.M., Germany. Culture of Pollacci, from Baarn.

2. CEPHALOSPORIUM STUEHMERI Schmidt & Beyma in Beyma, Centralbl. f. Bakt. I, **130**: 102–105, *3 figs.* 1933.
Pl. 2, figs. 17–21.

Colony: white to a pale pink, slightly pubescent above, little aerial growth, small white balls 1 to several mm. in diam. arising on elevated spots of colony in cultures 7–10 days old, about as thick as heavy paper; guttational water; reverse corrugated, pale orange, as is also adjacent medium; small coremia on Sabouraud's medium, and on carrot plugs a white pustular tallowy growth.

Conidiophores: unbranched, usually one to several septate, borne in a row along both sides of a straight hypha; sheaths of hyphae often with some strands protruding and bearing spores; phialides 35 × 1.5 μ; cephalosporia 10–15 μ; conidia ellipsoid, 4–5.5 × 2.5–3 μ, swelling slightly on germination, sprouting at one point and giving a mycelium that becomes early septate; vegetative cells 10 × 2 μ, uninucleate; submerged terminal chlamydospores 6.5–7 μ in diam., intercalary ones 7 μ.

Habitat: eczematoid infection of skin, Germany. Culture of Stuehmer, Baarn.

3. CEPHALOSPORIUM KILIENSE[3] (Gruetz) Hartmann, Derm. Woch. **82**: 569. 1926.
Pl. 2, figs. 26–31.

Acremonium kiliense Gruetz, Derm. Woch. **80**: 765–774. 1925.

C. asteroides griseum Gruetzii Benedek, Arch. f. Derm. & Syphilis **154**: 166. 1928.

C. Acremonium Pollacci & Nannizzi, I Miceti Pat. Uomo Anim. **9**: No. 81. 1930. Non Corda, 1839.

Colony: a few mm. thick, bittersweet-orange, finely corrugated, consisting of a number of small irregularly arranged strands, aerial growth slight, flat, and parallel; mycelium extending up on glass and consisting mainly of relatively thick cords upon which tiny cephalosporia can be seen with a lens;

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[3] See Dodge, Med. Myc. p. 828. 1935.

much guttational water; reverse slightly irregular, lighter-colored than upper surface; on Sabouraud's dextrose agar a faint white surface powder; on carrot plug an orange hairy coremial growth.

Conidiophores: along both sides of main stalk, especially clustered at ends and upon lateral branches where compound phialides are often borne in whorls of four or more, forming cephalosporia up to 14 μ in diam. in the air, or irregular non-slimy packets; phialides slightly tapering, 2–43 × 2.5 μ; conidia oblong-ellipsoid, hyaline, 2–4 × 1.5 μ; terminal chlamydospores 4–7 × 2–5 μ, intercalary ones 5 μ in diam.

Habitat: gummata and ulcers in man. Culture of Pollacci (?), from Baarn.

4. Cephalosporium Serrae Maffei, Atti Ist. Bot. R. Univ. Pavia IV, **1**: 183–198, *9 figs.* 1930. Pl. 2, figs. 1–11.

Colony: about 5 mm. thick, corrugated, at first white then sea-shell pink, in about ten days turning fuscous, aerial growth rough and coarse wooly, of short projections several mm. high, heaped, dry and partially wrinkled; a bit of fine growth on glass; no guttational water; reverse wrinkled and black; no color diffusing into medium; on carrot plug an olive-buff pustular glabrous growth with a few pink coremia.

Conidiophores: borne laterally, simple or once-seriate, of 3–5 phialides; main stalk 42 μ long; phialides somewhat tapering, 29 × 2.5 μ; cephalosporia 7.5 μ in diam.; conidia ellipsoid, 3–4 × 1.5 μ, sometimes not in a ball; chlamydospores 7.5 × 5 μ and 10 × 3.5 μ, terminally or on strands of upright coremia.

Habitat: keratomycosis of the eye. Culture of Pollacci, from Baarn.

5. Cephalosporium niveolanosum Benedek, Arch. f. Derm. & Syphilis **154**: 166. 1928. Pl. 2, figs. 12–16.

Colony: at first elevated, ivory-white, pasty, 5 mm. in diam., becoming a slightly pink, glistening, dry heap, edges flat with radial striations arranged turbinately (in the dark remaining ivory-white but becoming pink upon exposure to daylight); short delicate coremia at times; reverse convex from medium.

Conidiophores: simple, rare, septate or not; spores ellipsoid with two oil droplets, 6 × 3 μ; hyphal cells 20 × 2.5 μ; intercalary submerged chlamydospores 6.5 μ in diam., terminal thinner-walled ones, 15 μ.

Habitat: isolated from a case of dermatitis in Leipzig. Culture of Benedek, from Baarn.

6. CEPHALOSPORIUM **symbioticum**[4] Pinkerton, n. sp.

Pl. 2, figs. 32–38, 45–46.

Colony: apricot-buff, on wort agar sometimes becoming drab-green, with smooth surface except for small coremia, convoluted, growing into medium below; on liquid media producing a ring or pellicle, decolorizing litmus, coagulating milk, liquefying gelatine, no gas in sugars, producing acid in dextrose, d-levulose, raffinose, maltose, and sucrose, alkali in amygdalin, arabinose, d-galactose, lactose, and dextrin.

Within medium colony forms pseudomycelium of cells 4.0 × 2.5 μ, cephalosporia 8 μ in diam. at the apex of phialides, the latter 5 μ in length; aerial thallus a true mycelium, cells 8–10 × 2 μ; phialides 2–5 μ in diam.; spores 4–5 × 2.5 μ, some germinating in place. Mature colonies becoming fuscous with many terminal and intercalary chlamydospores 4–5 μ in diam.

Habitat: isolated from a severe case of dermatitis at the Barnard Free Skin and Cancer Hospital. It was intimately associated with *Torulopsis mucilaginosa* from which it was separated only by extreme dilution cultures. The subgenus *Cephaloblaston* of Ciferri ('32), typified by *C. pseudofermentum,* would also encompass this species.

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[4] CEPHALOSPORIUM **symbioticum**, sp. nov.

Colonia: "apricot-buff" aliquando "drab-green," in medio musto, superficie laevi praeter coremia parvo, convoluta, in media subter succrescens; in mediis liquidis annulum pelliculumve efficiens, litmo decolorans, lactem coagulans, gelatinam liquefaciens, in saccharis non effervescans; acidum in "dextrose, d-levulose, raffinose, maltose, sucrose" faciens, alcalinum in "amygdalin, arabinose, d-galactose, lactose, dextrin."

Colonia mycelium falsum intra medium formans, cellulis 4.0 × 2.5 μ, cephalosporiis 8 μ in diametro apice phialae 5 μ in longitudine; thallus aerius mycelium verum, cellulis 8–10 × 2 μ; phialae 2–5 μ in diametro; sporae 4–5 × 2.5 μ, aliquibus in situ germinantibus. In coloniis maturis fuscescentibus chlamydosporae multae terminales aut intercalares, 4–5 μ in diametro.

Case History.—The patient, a white female 15 years of age, first reported to the Barnard Free Skin and Cancer Hospital on April 2, 1934. She had a severe dermatitic eruption occurring in patches upon her hands, especially the fingers, and upon the forearms. The case was diagnosed as "dermatitis contact." Trichophytin tests were given and crude coal-tar salves advised. Various patent medicines previously used by the patient complicated the irritation. By August 31, 1934, there was a marked improvement, but on September 20, the condition was worse and X-ray treatments were given. On October 2, the hands showed many new vesicles and bullae and ruptured blisters which left raw spots. The condition was then diagnosed as a combination of tinea and *Staphylococcus* infection. She also suffered from acne and reported a history of scabies. In addition to X-ray treatments, a tincture of xeroform was recommended. The patient was last heard from on October 24, 1934.

Skin cultures included numerous organisms of which *Staphylococcus*, *Torulopsis mucilaginosa*, *Cephalosporium symbioticum*, and a black filamentous form (unidentified) were dominant. The *Cephalosporium* was non-pathogenic to white mice.

CLONOSTACHYS[5] Corda, Pracht-Flora, p. 31. 1839.

Creeping hyphae; conidiophores simple or branched; phialides four or more branched, verticillate; spores adhering in cephalosporia. Type: *C. Araucaria* Corda.

1. CLONOSTACHYS ARAUCARIA Corda, Pracht-Flora, p. 31. 1839. Pl. 5, figs. 14–26.

Colony: white-cottony, soon turning pale flesh to seashell pink, powdery, rapidly growing; coarse white-pink coremia in older cultures; mycelium extending up glass for several mm., bearing cephalosporia; much guttational water; reverse wrinkled. Species differs superficially from the former *Gliocladium roseum* in pinker color, slower growth, less extensive coremia formation, and producing acid in maltose.

Conidiophores: polymorphic, some of the characteristic of four-branched type; asymmetrical penicillate fructifications formed on reversed wort plates with terminal clusters of six phialides and laterals of three to six, phialides 10 × 2 μ, flask-shaped and bent outwards, conidia spherical to ellipsoid, 3 × 1–2 μ; cephalosporia borne along the main cord, singly or in clusters of three to six, phialides slightly flask-shaped, 17–33 × 1.5–4 μ; cephalosporia 13 μ, conidia 5.5–6.5 × 2.5–3 μ; hyphae warty, especially near cephalosporia; chlamydospores rare, on corn meal agar in terminal series, 13 × 10 μ; or on lactose broth intercalary, 10 × 12.5 μ; hyphal cells 13 × 3 μ.

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[5] The investigation of the type species and varieties permits enlargement upon Corda's original limited description. Unfortunately, the author is unfamiliar with the other species usually included here, but because of the limited generic description it is doubtful if they form a homogeneous group. The type, which has been inadequately characterized, does seem sufficiently different to justify its position in a genus intermediate between *Cephalosporium* and *Gliocladium*. *Clonostachys spectabilis*, which was studied only briefly due to an early demise, obviously is far removed and certainly suggests *Botrytis*. Presumably Oudemans ('86) compared the botryoid spores to the lateral protrusions sometimes found on *C. Araucaria* and its varieties, which are not spores.

Habitat: "In cortice ramulorum arborum in Germania, Brittania" (Corda). Culture of Wakefield, from Baarn.

2. CLONOSTACHYS ARAUCARIA var. ROSEA Preuss, Linnaea **25**: 727. 1852. Pl. 4, figs. 27–31.

Colony: salmon-pink almost at first, mottled with white, floccose, of strands several mm. long, heaped in center and irregularly wrinkled, coarsely flocculent at edges; much guttational water; reverse wrinkled. Variety differs from the former *Gliocladium roseum* and the species in the salmon tint, intermediate speed of growth, and is more wrinkled than either.

Conidiophores: mostly typical of four phialides as illustrated by Corda, the spores from several phialides sometimes forming confluent slime balls; cephalophores up to 100 μ; phialides non-septate, 17–40 × 6 μ; cephalosporia 21 μ in diam.; conidia 4 × 5 μ; chlamydospores terminal and intercalary, 3.5 × 10 μ.

Habitat: "In ramulis, in piris et pomis immaturis dejectis, frequens, Hoyerswerda" (Preuss). Culture of Shapovalov, from Baarn.

3. CLONOSTACHYS ARAUCARIA var. **confusa** Pinkerton, n. var. Pl. 4, figs. 15–26.

Penicillium roseum Link (?), Ges. Naturforsch. Fr. Berlin, Mag. **7**: 37. 1816.

Gliocladium roseum Bainier, Bull. Soc. Myc. France **23**: 111–112, *pl. XV, figs. 1–6.* 1907. Nec *Clonostachys Araucaria* var. *rosea* Preuss.

Colony: pale ochraceous salmon, slightly mottled with white, short-cottony and mealy on top, growing up tube very little, slightly adherent; on inverted Petri plate of wort agar producing white feathery extensions measuring up to 1 cm. long; much guttational water; reverse much wrinkled.

Conidiophores: polymorphic; penicillate conidiophores asymmetrical with branches terminating in whorls of three to four, penicillus 85 μ high, main lateral segments 45 μ, phialides 8–10 × 1.5–2 μ, flask-shaped, mature conidia spherical to slightly falcate, 5 μ, swelling on germination to 8–10 μ, growing from one or both poles; spore balls from single phialide along main hyphae or rope, or usually in whorls of three, borne laterally or terminally, phialides 30–40 × 3–4 μ, warty, straight or slightly narrower at top, adjacent balls sometimes becoming confluent and reaching 10–30 μ in diam., conidia ellipsoid, smooth, hyaline, pink in mass, 6–7 × 4–5.5 μ; chlamydospores rarely formed but found terminally on submerged hyphae on Raulin's agar, 4.5 μ in diam., also on inverted wort plates, intercalary ones 14 × 20 μ.

Habitat: "In caule solani tuberosi exsiccati. Misit Dr. Nees ab Esenbeck." (Link); "du carton pourri" (Bainier). Culture from Thom No. 454–4640.428, from Kral in Prague, Bohemia.

Bainier stated that this form resembled *Acrostalagmus roseus* of the Verticillieae except that the precedence of penicillate over verticillate series was reversed in *Gliocladium.* More probably the variations are dependent upon nutrient relations, especially moisture conditions. On lactose broth hanging drops, the spore balls are most luxuriant, as are also the warts. Complex penicillate fructifications are also present, however, and in fact precede the simpler cephalosporial stage both in time and on the main trunk. The spores on the penicillus cling together only loosely in irregular chains as seen on slide cultures, and are quickly disseminated in water. Conspicuous deliquescence of the penicillate heads, as occurs in *G. deliquescens,* was not noted. The fact that in crossing this form is antagonistic to the species, whereas the rest are all indifferent, might indicate a close relationship of a lethal nature.

4. CLONOSTACHYS SPECTABILIS (Harz) Oudem. & Sacc. Ned. Kruidk. Arch. II, **4**: 539. 1886. Pl. 5, figs. 1–7.

Botrytis spectabilis Harz, Bull. Soc. Imp. Nat. Moscow **44**: 88–147. 1871.

Original colony gray-white and fuzzy, turning black due to production of numerous conidia.

Conidiophores compound, dichotomously branched and bearing spores on short stalks laterally along the phialides; spores spherical, 6.5–7 μ in diam.; chlamydospores 6.5–10 μ in diam.

Habitat: "sur la tannée dans les serres du jardin botanique d'Amsterdam" (Balen). Culture of Shear, from Baarn.

GLIOCLADIUM[6] Corda, Icon. Fung. **4**: 30–31. 1840.

Conidiophores erect, septate, penicillately branching above, branches and branchlets septate, appressed, forming a solitary gelatinous head; conidia unicellular, borne upon the tips of branchlets and held together by mucilaginous substance in a dense mass, those on adjacent phialides fusing. Type: *G. penicilloides* Corda.

1. GLIOCLADIUM FIMBRIATUM Gilman & Abbott, Iowa State Coll. Jour. Sci. **1**: 304, *fig. 38*. 1927. Pl. 3, figs. 33–41.

Colony: at first white, fuzzy, nearly circular, very thin, moist, translucent with parallel wrinkles, finally antique green, mottled with white, dry, floccose, faintly zonate and radially striate, broad masses of white hyphae growing up tube several cm.; reverse radially corrugate.

[6] The inclusion of the former *Gliocladium roseum* in *Clonostachys* might possibly also justify lumping *Gliocladium* in that genus whose publication preceded it by one year. However, the three sections of *Gliocladium* mentioned by Thom ('30)—the rose, white, and green series—seem quite distinct culturally and morphologically. There is one common character to the two genera, i. e., the lack of disjunctors and presence of cephalosporia, which in view of the heterogenous mass included under *Penicillium* might make such a segregation logical.

Gliocladium penicilloides resembles *Clonostachys Araucaria* in a rather similar zonate growth and exudation of yellow color into medium, but differs in the thin-walled auxiliary cells (akinetes). *G. Vermoeseni* is quite distinct in its colonial characters and the spore type which approaches *Penicillium* in its rigidness, but still lacks disjunctors. *G. deliquescens* and *G. fimbriatum* are very different from each other as well as the others in colonial characters but have akinetes. Therefore, due to insufficient knowledge of the *Gliocladium* and *Clonostachys* segregates as a whole, they will not be combined here.

Conidiophores: homogeneous, borne laterally and terminally in a wide range of heights up to 300 μ, more or less symmetrical in two series, basal cell 14.5 × 5.3 μ; first series 6.5 × 4 μ; phialides very turgid and asymmetrically flask-shaped, 8.5 × 4.5 μ; spores ellipsoid, 4.5 × 3.3 μ, forming irregular spore balls up to 16.5 μ in diameter, dry and only slightly mucoid; akinetes terminal and intercalary, frequent, 11–13 × 8.5–10 μ, containing oil droplets up to 1.3 μ, these soluble in chloroform leaving striations; chlamydospores intercalary, 9–9.5 × 8 μ.

Habitat: in soil, Iowa and Louisiana. Culture of Gilman & Abbott, from Thom No. 459–4894.20.

2. GLIOCLADIUM DELIQUESCENS Sopp, Monogr. pp. 89–93, *taf. I, fig. 1–6.* 1912. Pl. 3, figs. 20–32.

Colony: at first colorless, of mainly submerged hyphae, quickly becoming thin and white-floccose over whole surface, then meadow-green and slimy, progressing slowly centripetally from edge; much guttational water; very rapid growth of hyaline semi-submerged radiating hyphae.

Conidiophores: of two types—penicillate and simple cephalosporial; penicilli at first white but spores become green and aggregate in a heavy slime, typically tri-seriate and each series trichotomous; brush 45 × 40 μ; first series 17.5–24 × 4 μ; second series 9–11 × 4 μ; phialides 6–9 × 2–2.5 μ, spores smooth, spherical to ellipsoid, 3.5 × 2.5 μ, germinating spores swelling up to 6.5–9 μ, sprouting in one or more places; spore balls up to 30 μ; cephalosporia up to 60 μ, circular with thick matrix of slime, cephalolides 40 × 6 μ; conidia 2 μ in diam.; akinetes 7.5–10 × 6.5–8.5 μ, terminal or intercalary; pseudoterminal ovoid cells 14 × 11.5 μ; chlamydospores 6 × 7.5 μ.

Habitat: on *Daedalea unicolor* in Norway. Culture of Thom No. 457–4894.17.

3. GLIOCLADIUM VERMOESENI (Biourge) Thom, The Penicillia, p. 502. 1930. Pl. 4, figs. 1–14.

Penicillium Vermoeseni Biourge, La Cellule **33**: 230. 1923.

Colony: pale vinaceous, rapidly growing, irregularly powdery and with thin long hairs; much guttational water; reverse homogeneous and when viewed through appears splotchy.

Conidiophores: usually penicillate, 45 μ long from first branch, decidedly asymmetric, poly-seriate, final series trichotomous; phialides up to 10 × 1.5–3.5 μ; spores spherical, 2.5 μ; cephalolides 40 μ and more, cephalosporia 20–25 μ, cephalospores ellipsoid, 5–6.5 × 4 μ; intercalary chlamydospores 13 × 15.5 μ, terminal 5 × 11 μ; akinetes 15 μ in diam.; arthrospores 10.5 × 5 μ; oidia 3 × 5.5 μ, rare.

Habitat: parasitic or semi-parasitic on certain species of *Areca*. Culture of Westerdijk, from Thom No. 451–5090.4a.

4. GLIOCLADIUM PENICILLOIDES[7] Corda, Icon. Fung. **4**: 31, *taf. 7. figs. 88–89.* 1840. Pl. 3, figs. 1–19.

Colony: at first chalky-white, of short regularly centrifugally appressed hairs, faintly and scarcely striate, within a month very dry and tan to light pink. On glycerine agar sometimes producing a lemon-yellow pigment which diffuses into the medium; growth relatively slow and consistent.

Conidiophores: asymmetric, often borne as more or less short laterals along main branch, up to 114 μ long, septate; phialides 17 × 3 μ, slightly flask-shaped, borne in threes, occasionally fours or more, of which one is larger; lowest branches alternate, usually not more than three main divisions; spores ellipsoid, 5.5–6.5 × 3 μ, swelling before germination to 8 × 5 μ; single branched phialides, septate or not, 35.5 × 2.5 μ, bearing cephalosporia 14 μ in diam.; spores 4 × 2.5 μ; vegetative cells 3.5–5 μ in diam., racquet-shaped, consisting of several large vacuoles; akinetes 10 μ in diam., full of oily reserves and capable of almost immediate germination.

Habitat: on rotting *Thelephora*. Culture of Thom No. 453–5113.

SCOPULARIOPSIS Bainier, Bull. Soc. Myc. France **23**: 98. 1907.

Type: *Penicillium brevicaule* Saccardo.

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[7] Winter ('87) suggested that probably *Eurotium insigne* Winter was the ascosporic form of this species. Matruchot ('95) found perithecia of the Plectascales type, but through the unique method of ascospore germination distinguished it from *E. insigne;* however, he offered no other nomenclatorial suggestion. Theoretically, the species should be removed to the perfect classification, but for the present such shall not be attempted.

1. SCOPULARIOPSIS BREVICAULIS (Sacc.) Bainier, Bull. Soc. Myc. France **23**: 99–103, *pl. 11, figs. 1–6.* 1907. Pl. 5, figs. 8–13.

Penicillium brevicaule Sacc. Fung. Ital. *taf. 893.* 1881.

White flocculent growth upon glycerine agar.

Conidiophores simple or few-branched; phialides 8 × 2–3 μ; spores 5 μ in diam. with disjunctors between, adhering in chains indefinitely; no mucus; no chlamydospores noted.

Habitat: one strain reported as pathogenic by Dr. D. J. Davis of Chicago. Culture of Thom No. 4858.

This species was studied only very superficially as living material, in an attempt to correlate the presence of disjunctors and no slime with the lack of disjunctors and mucus, also to note methods of phialide mechanism.

TORULOPSIS Berlese, Giorn. Viticoltura ed Enologia p. 54. 1894.

Type: *T. rosea* Berlese.

TORULOPSIS MUCILAGINOSA (Jörgensen) Ciferri & Redaelli, Atti Bot. Ist. R. Univ. Pavia III, **2**: 256. 1925.

Pl. 2, figs. 39–44, 47.

Torula mucilaginosa Jörgensen, Die Mikroorg. d. Gärungsindustrie. ed. 5, 402. 1909.

Rhodotorula mucilaginosa Harrison, Trans. Roy. Soc. Canada **22**: 187. 1928.

Cryptococcus rubrorugosus Castellani, Arch. of Derm. & Syphilol. **16**: 403. 1927.

Rhodotorula mucilaginosa race *rubrorugosa* Lodder, Anaskosporogenen Hefen **1**: 104–105. 1934.

Colony: Carnelian red to scarlet, moist and smooth, pasty, rapidly growing upon wort and maltose agar, with elliptical cells showing bipolar budding and a pair of oil droplets in mature cells; on nitrate medium, following the sparse red surface growth, a submerged irregular hyaline one of pseudomycelial cells and small asymmetrical cephalosporia up to 15 μ sometimes occurring, spores 2.5 × 5 μ; in malt broth a ring, some red basal proliferation and slight pseudomycelial growth throughout liquid; slight growth in ethyl alcohol; ring in grape juice but no fermentation, no liquefaction of gelatin, milk clotted; no gas in sugar solutions; acid produced in sucrose, d-levulose, maltose and dextrose, alkali in arabinose, amygdalin, d-galactose, lactose, raffinose and dextrin.

Habitat: originally isolated as a laboratory contaminant; Castellani reported it from scrapings in the axilla; and it was secured by the author from skin scrapings in a severe dermatomycosis.

VIII. Summary

1. The term cephalophore has been applied to a conidial fructification wherein the spores are held in a spherical mass by mucilage. These spores are found to be produced endogenously by cleavage following exudation of liquid which forms the matrix of the spore ball. Terminal granules seem to be kinetic centers associated with exudation and spore discharge. The phialides are thought to be uninucleate as are also the spores.

2. Accessory spore forms include chlamydospores in all cases, associated both with the aerial and submerged mycelium, oidia infrequently, and thin-walled spores in some, which have been called akinetes after the algological terminology.

3. An attempt has been made to correlate comparative cultural data regarding color, macroscopic and microscopic characters. It is believed that such factors as hydrogen-ion concentration, surface tension, oxygenation, and the nature of the nutrients are especially important in producing favorable or unfavorable conditions for the organism. Good growth is characterized by abundance of aerial mycelia with a considerable pigmentation; and poor growth, by numerous spore-forms, attenuated aerial hyphae with proportionally more reproductive bodies and a tendency towards submerged growth and exudation of pigments into the medium, and a paler color of the organism. *Torulopsis mucilaginosa* develops a pseudomycelium in the submerged state and loses its red color.

4. Mycelial pigmentation is noted in some cases to be more intense under optimal conditions; in some to be light labile. The solubilities of the pigment of *Cephalosporium kiliense* are listed, although no identification is made.

5. Polarized light indicates no structural difference between the walls of the phialides and of the mycelial cells.

6. Various unsuccessful attempts have been made to produce sexual fructifications. Theories regarding loss of sexuality are discussed, but no further contributions are suggested.

7. The clinical findings of some of the organisms in dermatomycoses are mentioned, although their pathogenicity has not been proven. The granular incrustations upon serum are described. These were found to be composed of fine needle-like crystals arranged radially and including no recognizable organic matrix but surrounded by fine mycelial filaments. The crystals were slowly soluble in the concentrated heavy acids. The theoretical significances of these structures are discussed.

8. A brief morphological description is given of all the forms studied. *Gliocladium roseum* (Link?) Bainier is described as *Clonostachys Araucaria* var. *confusa*. The position of *Gliocladium penicilloides* is discussed. The new species isolated from the dermatomycosis case at the Barnard Free Skin and Cancer Hospital is designated as *Cephalosporium symbioticum.*

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Explanation of Plate

PLATE 1

All drawings, except fig. 17, made with the aid of a camera lucida.

Fig. 1. Mature compound cephalosporium in *Clonostachys Araucaria.* Nutrient broth. × 750.

Fig. 2. Incipient phialide in *Gliocladium penicilloides.* Glycerine agar. × 750.

Figs. 3–4. Basophilic granules in phialides of *G. penicilloides.* Glycerine agar. × 1250.

Fig. 5. Exudation of mucilage in *Clonostachys Araucaria.* Nutrient broth hanging drop. × 1250.

Fig. 6. Phialide enclosed in mucilage in *C. Araucaria.* Endo's agar. × 750.

Fig. 7. Orientation of granules at tips of phialides, and cleavage vacuoles in *Gliocladium penicilloides.* Glycerine agar. × 1250.

Fig. 8. Splitting of phialide tip in *G. penicilloides.* Glycerine agar. × 1250.

Figs. 9–10. Emergence of conidia in *G. penicilloides.* Glycerine agar. × 1250.

Fig. 11. Endoconidia in *G. penicilloides.* Glycerine agar. × 1250.

Fig. 12. Twisted phialide with uninucleate endoconidia in *G. penicilloides.* Glycerine agar. × 1250.

Fig. 13. Mature uninucleate conidium of *G. penicilloides.* Glycerine agar. × 1250.

Fig. 14. Conidiophores with endoconidia, and bi-vacuolate spores in *Clonostachys Araucaria* var. *confusa.* Litmus milk. × 1250.

Fig. 15. Phialides of *Gliocladium penicilloides* from paraffin sections at 3 μ. Glycerine agar. × 1250.

Fig. 16. Constriction of spore mass during exit in *G. penicilloides.* Litmus milk. × 1000.

Fig. 17. Diagram showing successive production of conidia, from observations at the end of 10, 20, 35, 65, and 70 minutes in *Clonostachys Araucaria.* Sabouraud's dextrose agar.

Figs. 18–28. *Allescheria Boydii.*

Fig. 18. Submerged chlamydospore. Nitrate agar. × 750.

Fig. 19. Conidium. Raulin's agar. × 750.

Figs. 20–21. Stages of development of submerged cephalosporia. Sabouraud's dextrose agar. × 350.

Fig. 22. Coremium stage. Sabouraud's dextrose agar. × 1250.

Fig. 23. Submerged lateral spore. Raulin's agar. × 1250.

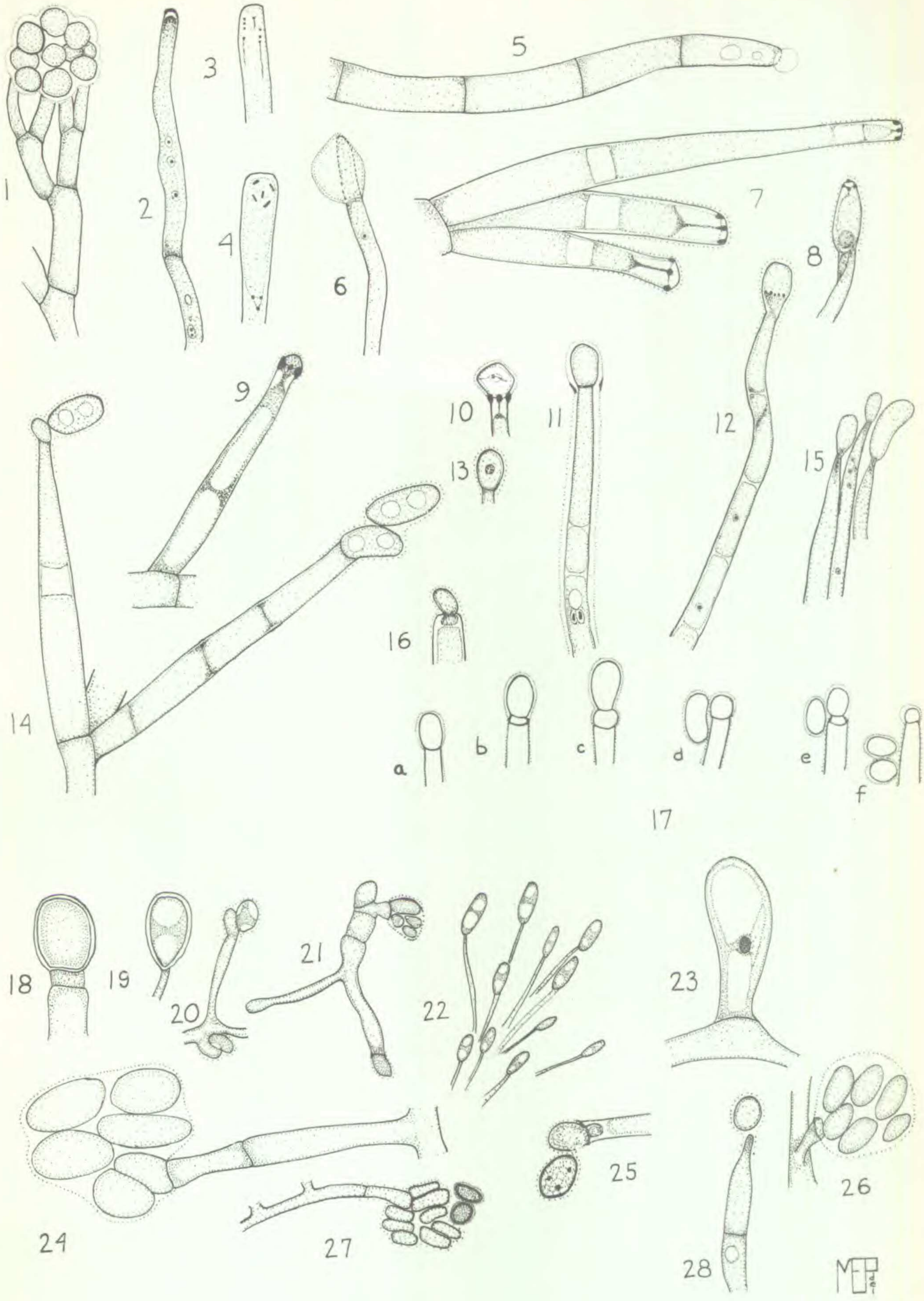
Fig. 24. Cephalosporium. Sabouraud's dextrose agar. × 1250.

Fig. 25. Mature and young spore. Sabouraud's dextrose agar. × 350.

Fig. 26. Lateral cephalosporium. Sabouraud's dextrose agar. × 1250.

Fig. 27. Young and mature spores in cephalosporium. Raulin's agar. × 350.

Fig. 28. Conidiophore. Raulin's agar. × 1250.



PINKERTON — CONIDIAL FORMATION

COCKAYNE, BOSTON

Explanation of Plate

PLATE 2

Unless otherwise stated, all drawings were made with the aid of a camera lucida.

Figs. 1–11. *Cephalosporium Serrae.*
- Fig. 1. Terminal chlamydospore. Potato agar. × 1250.
- Fig. 2. Series of chlamydospores. Potato agar. × 750.
- Fig. 3. Chlamydospores. Corn meal agar. × 750.
- Fig. 4. Cephalosporium within medium. Raulin's agar. × 1250.
- Fig. 5. Twisted phialide with endoconidia. Sabouraud's dextrose agar. × 1250.
- Fig. 6. Phialide with vacuoles. Sabouraud's dextrose agar. × 1250.
- Fig. 7. Dimorphic cephalosporium. Maltose agar. Not camera lucida.
- Fig. 8. Diagram of fructification.
- Fig. 9. Germinating chlamydospore. Nutrient broth. × 750.
- Fig. 10. Mucilage drop. Nutrient broth. × 750.
- Fig. 11. Packet type of cephalosporium. Nutrient broth. × 750.

Figs. 12–16. *Cephalosporium niveolanosum.*
- Fig. 12. Terminal chlamydospore. × 750.
- Fig. 13. Chlamydospores. Corn meal agar. × 750.
- Fig. 14. Phialide with endoconidia. Nutrient agar. × 750.
- Fig. 15. Phialide producing spore. Nutrient agar. × 750.
- Fig. 16. Young cephalosporium. Nutrient agar. × 750.

Figs. 17–21. *Cephalosporium Stuehmeri.*
- Fig. 17. Conidiophore with endoconidium. Sabouraud's dextrose agar. × 750.
- Fig. 18. Diagram of fructification.
- Fig. 19. Terminal chlamydospore. Corn meal agar. × 750.
- Fig. 20. Chlamydospores. Sabouraud's dextrose agar. × 350.
- Fig. 21. Peculiar aerial spore. Sabouraud's dextrose agar. × 350.

Figs. 22–25. *Cephalosporium rubrobrunneum.*
- Fig. 22. Origin of phialides from ropes of hyphae. Glycerine agar. × 350.
- Fig. 23. Slanted spore cluster. Sabouraud's dextrose agar. × 350.
- Fig. 24. Young cephalosporium. Sabouraud's dextrose agar. × 350.
- Fig. 25. Uninucleate spore. Sabouraud's dextrose agar. × 350.

Figs. 26–31. *Cephalosporium kiliense.*
- Fig. 26. Diagram of fructification. Endo's agar.
- Fig. 27. Cephalosporium. × 350.
- Fig. 28. Packet cephalosporium. Sabouraud's dextrose agar. Not camera lucida.
- Fig. 29. Cephalosporium. Sabouraud's dextrose agar. × 350.
- Fig. 30. Chlamydospores. Corn meal agar. × 1250.
- Fig. 31. Chlamydospores. Malt extract broth. × 1250.

Figs. 32–38. *Cephalosporium symbioticum.*
- Fig. 32. Chlamydospore. Glycerine agar. × 750.
- Fig. 33. Conidium. Nutrient broth. × 1250.
- Fig. 34. Cephalosporia. Endo's agar. × 600.
- Fig. 35. Conidia. Nutrient agar. × 1250.
- Fig. 36. Young submerged growth. Sabouraud's dextrose agar. × 1250.
- Fig. 37. Submerged growth on wort agar. × 1250.

EXPLANATION OF PLATE

PLATE 2 *(Continued)*

Fig. 38. Chlamydospores. Sabouraud's dextrose agar. × 1250.

Figs. 39–44. *Torulopsis mucilaginosa.*

Fig. 39. Resting cell. Malt extract broth. × 750.

Fig. 40. Bipolar budding. Malt extract broth. × 750.

Fig. 41. Submerged pseudomycelium. Nitrate agar. × 750.

Fig. 42. Pseudomycelium and irregular spore clusters. Endo's agar. × 600.

Fig. 43. Submerged cephalosporium. Nitrate agar. × 750.

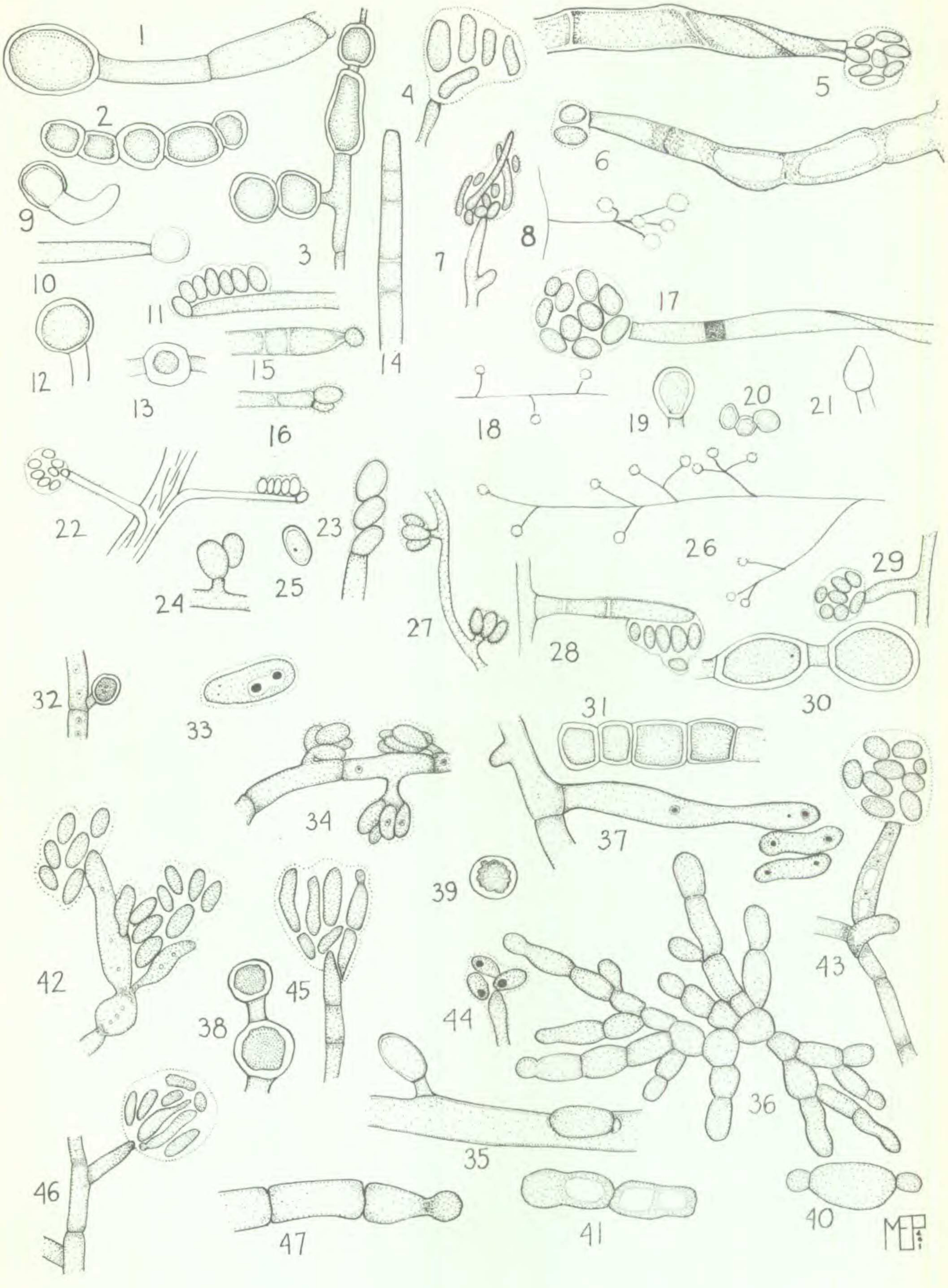
Fig. 44. Submerged sporiferous tip. Nitrate agar. × 750.

Figs. 45–46. *Cephalosporium symbioticum.*

Fig. 45. Cephalosporium with spore germinating *in situ.* Wort agar. × 1250.

Fig. 46. Cephalosporium. Wort agar. × 1250.

Fig. 47. Submerged mycelium of *Torulopsis mucilaginosa,* forming new cell by constriction. × 1250.



PINKERTON — CONIDIAL FORMATION

COCKAYNE, BOSTON

Explanation of Plate

PLATE 3

Drawings made with the aid of a camera lucida.

Figs. 1–19. *Gliocladium penicilloides.*

Fig. 1. Conidiophore. Glycerine agar. × 350.

Fig. 2. Diagram of fructification.

Fig. 3. Cephalosporium. Nutrient broth. × 350.

Fig. 4. Cephalosporia. Nutrient broth. × 600.

Fig. 5. Phialides with basophilic granules. Glycerine agar. × 1250.

Fig. 6. Phialide with mature spore and endoconidia. Glycerine agar. × 1250.

Fig. 7. Phialide with broken tip. Glycerine agar. × 1250.

Fig. 8. Phialide with basophilic granules. Glycerine agar. × 1250.

Fig. 9. Phialide with basal vacuole. Glycerine agar. × 1250.

Fig. 10. Conidium. Glycerine agar. × 1250.

Fig. 11. Conidia showing granular material. Glycerine agar. × 1250.

Fig. 12. Conidia. Glycerine agar. × 1250.

Fig. 13. Germinating conidium. Glycerine agar. × 750.

Fig. 14. Fusions of germ tubes of conidia. Nutrient broth. × 350.

Fig. 15. Akinete. Glycerine agar. × 750.

Fig. 16. Germinating akinete. Glycerine agar. × 750.

Fig. 17. Hypha. Glycerine agar. × 1250.

Fig. 18. Hyphal fusions. Nutrient broth. × 1250.

Fig. 19. Sub-phialide cell. Glycerine agar. × 1250.

Figs. 20–32. *Gliocladium deliquescens.*

Fig. 20. Submerged mycelial cell showing granular bodies at septa. Glycerine agar. × 1250.

Fig. 21. Tip of mycelial cell with large dancing bodies and small terminal granules. Nutrient broth. × 1250.

Fig. 22. Young conidiophore. Glycerine agar. × 1000.

Fig. 23. Cephalosporium. × 350.

Fig. 24. Diagram of cephalosporium branching structure.

Fig. 25. Conidia with chromatic material. Glycerine agar. × 1250.

Fig. 26. Conidia from smear stained with gentian violet. × 1250.

Fig. 27. Aerial hypha with granular tip. Glycerine agar. × 1250.

Fig. 28. Chlamydospore (?). Sabouraud's dextrose agar. × 800.

Fig. 29. Chlamydospores. Glycerine agar. × 1000.

Fig. 30. Aborted ascogonium (?). Glycerine agar pH 3.8. × 350.

Fig. 31. Germinating akinete. Sabouraud's dextrose agar. × 750.

Fig. 32. Phialide with terminal granules. Glycerine agar. × 1250.

Figs. 33–41. *Gliocladium fimbriatum.*

Fig. 33. Germinating conidium. Endo's agar. × 600.

Fig. 34. Chlamydospores. Sabouraud's dextrose agar. × 600.

Fig. 35. Conidiophore. Corn meal agar. × 750.

Fig. 36. Dimorphic fructification. Sabouraud's dextrose agar. × 350.

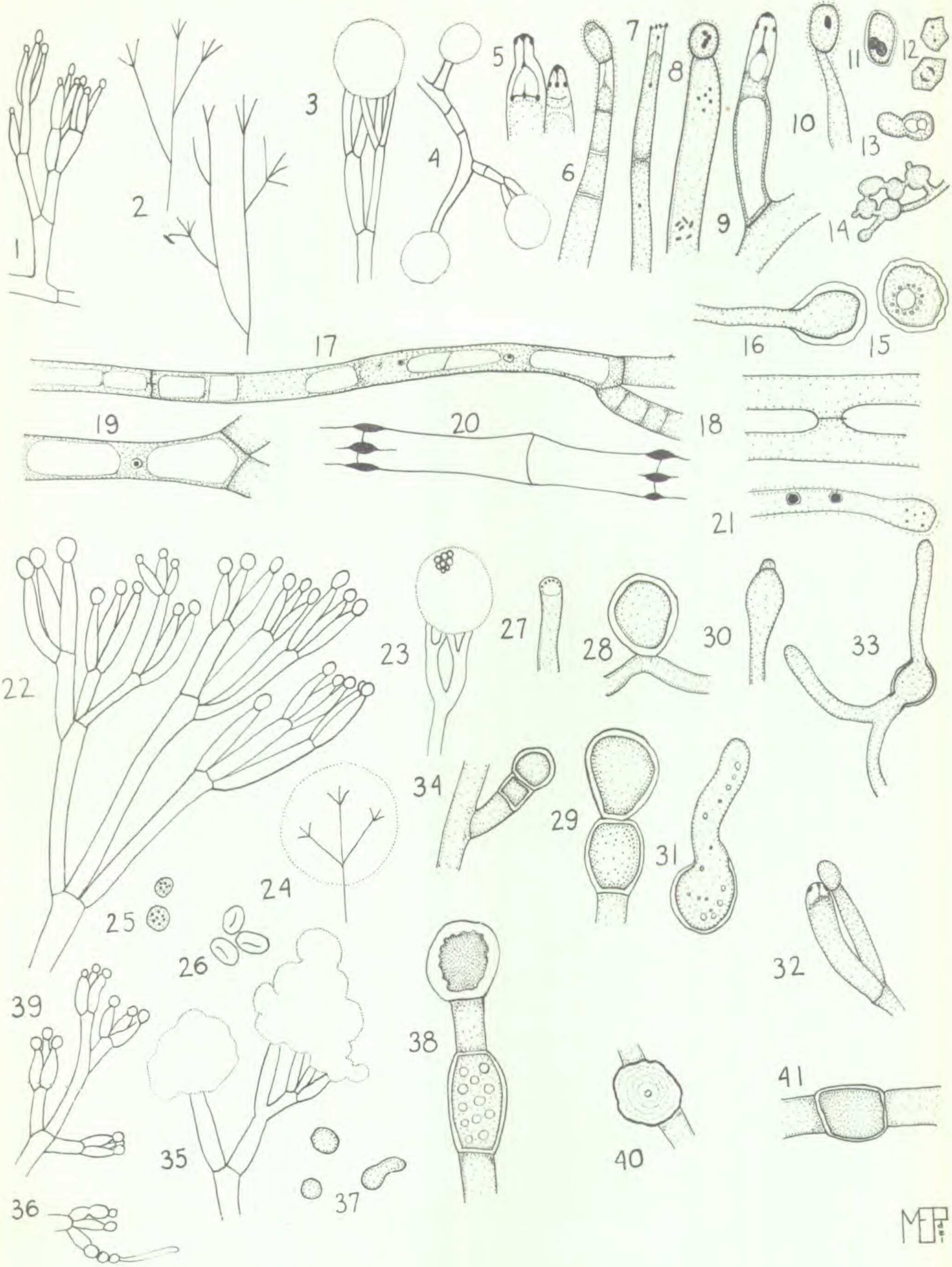
Fig. 37. Conidia. Sabouraud's dextrose agar. × 750.

Fig. 38. Akinetes. Glycerine agar. × 750.

Fig. 39. Conidiophore. Sabouraud's dextrose agar. × 350.

Fig. 40. Akinete upon addition of alcohol and chloroform, showing dissolution of oil. × 750.

Fig. 41. Intercalary chlamydospores. Corn meal agar. × 750.



PINKERTON — CONIDIAL FORMATION

CKAYNE, BOSTON

Explanation of Plate

PLATE 4

Drawings made with the aid of a camera lucida.

Figs. 1–14. *Gliocladium Vermoeseni.*

Fig. 1. Submerged chlamydospores. Sabouraud's dextrose agar. × 350.

Fig. 2. Aerial chlamydospores. Raulin's agar. × 750.

Fig. 3. Endoconidial method of formation of chlamydospores. Sabouraud's dextrose agar. × 750.

Fig. 4. Arthrospores. Corn meal agar. × 750.

Fig. 5. Conidiophore. Sabouraud's dextrose agar. × 750.

Fig. 6. Mature conidium. Sabouraud's dextrose agar. × 750.

Fig. 7. Part of cephalosporium showing exterior thick granular layer of mucus and spores deformed by pressure. Sabouraud's dextrose agar. × 750.

Fig. 8. Branch of penicillate fructification. Czapek's agar. × 350.

Fig. 9. Conidiophores. Corn meal agar. × 750.

Fig. 10. Unusual conidiophore. Raulin's agar. × 750.

Fig. 11. Oidia (?). Raulin's agar. × 750.

Fig. 12. Anomalous structure. Raulin's agar. × 1250.

Fig. 13. Diagram of fructification.

Fig. 14. Submerged akinete. Raulin's agar. × 750.

Figs. 15–26. *Clonostachys Araucaria* var. *confusa.*

Fig. 15. Conidiophore. Czapek's agar. × 350.

Fig. 16. Conidiophore with warts. Czapek's agar. × 350.

Fig. 17. Short lateral cephalosporium. Sabouraud's dextrose agar. × 350.

Fig. 18. Phialides with endoconidia. Endo's agar. × 1000.

Fig. 19. Chlamydospore (?). Sabouraud's dextrose agar. × 600.

Fig. 20. Dimorphic fructification. Lactose broth. × 600.

Figs. 21–22. Copulating conidia. Litmus milk. × 1250.

Fig. 23. Mature conidium. × 750.

Fig. 24. Germinating conidium. Sabouraud's dextrose agar. × 750.

Fig. 25. Hyphal fusions. Endo's agar. × 350.

Fig. 26. Submerged chlamydospore. Raulin's agar. × 750.

Figs. 27–31. *Clonostachys Araucaria* var. *rosea.*

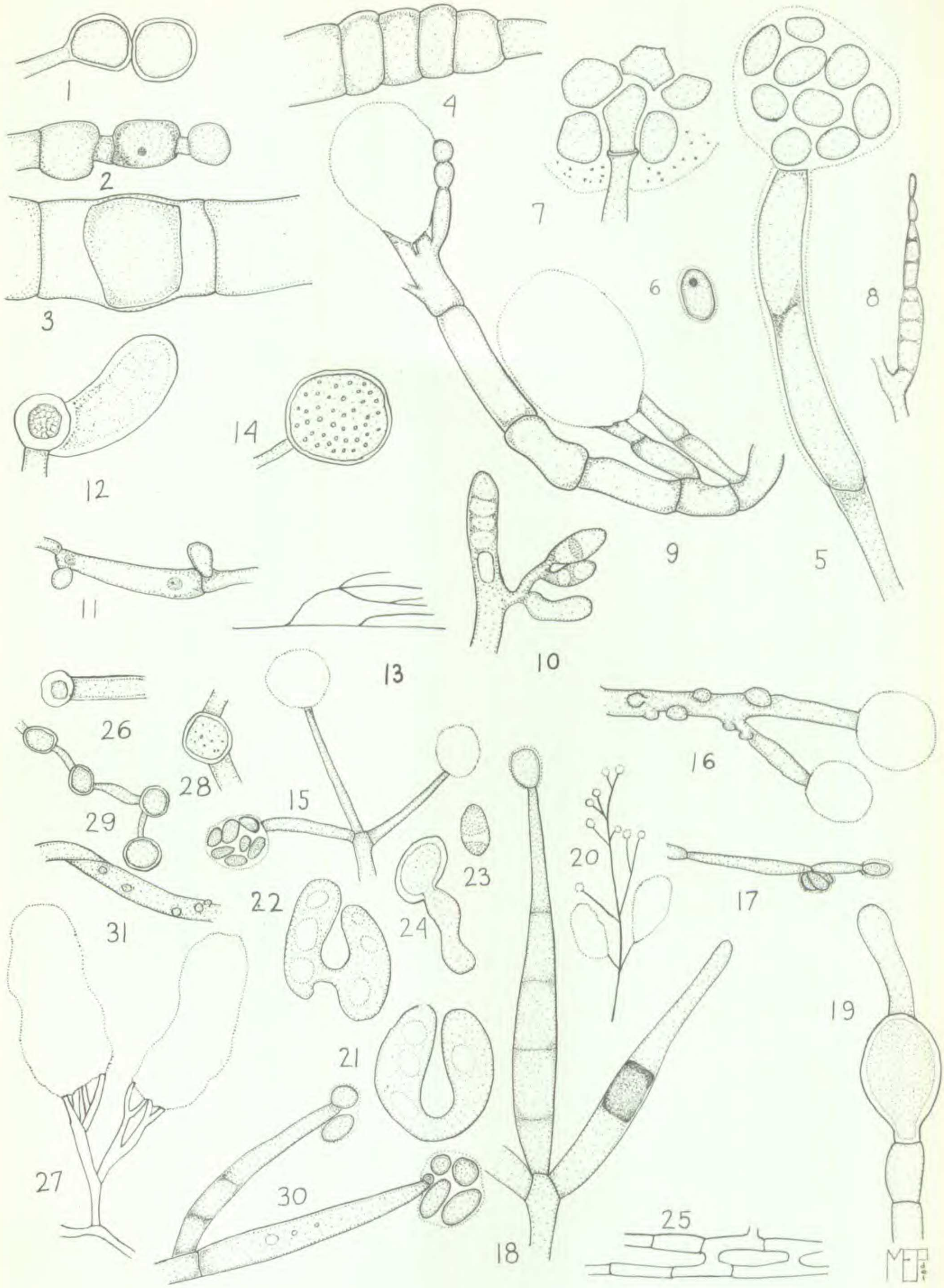
Fig. 27. Cephalosporia. Endo's agar. × 250.

Fig. 28. Chlamydospore. Corn meal agar. × 750.

Fig. 29. Submerged chlamydospores (?). Wort agar. × 350.

Fig. 30. Phialides with endoconidia. Sabouraud's dextrose agar. × 750.

Fig. 31. Hypha with warts. Sabouraud's dextrose agar. × 750.



PINKERTON — CONIDIAL FORMATION

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Explanation of Plate

PLATE 5

Drawings made with the aid of a camera lucida.

Figs. 1–7. *Clonostachys spectabilis.*

Fig. 1. Terminal chlamydospores. Cherry agar. × 750.

Fig. 2. Intercalary chlamydospore. Malt extract broth. × 750.

Fig. 3. Submerged terminal cell (?). Malt extract broth. × 750.

Fig. 4. Botrytis type of spore. Sabouraud's dextrose agar. × 350.

Fig. 5. Same. Corn meal agar. × 350.

Fig. 6. Mycelium associated with Botrytis type of fructification. Czapek's agar. × 350.

Fig. 7. Spores. Corn meal agar. × 750.

Figs. 8–13. *Scopulariopsis brevicaulis.*

Fig. 8. Young conidium before putting down endogenous wall. Nutrient agar. × 1250.

Fig. 9. Mature conidium and phialide showing segmentation. Nutrient agar. × 1250.

Fig. 10. Conidium. Nutrient agar. × 1250.

Fig. 11. Branched conidiophore. Nutrient broth. × 1250.

Fig. 12. Phialide with conidia. Nutrient agar. × 1250.

Fig. 13. Conidia. Nutrient agar. × 1250.

Figs. 14–26. *Clonostachys Araucaria.*

Fig. 14. Chlamydospores. Corn meal agar. × 750.

Fig. 15. Mycelium with warts. Sabouraud's dextrose agar. × 750.

Fig. 16. Dimorphic conidiophore. × 350.

Fig. 17. Cephalosporium and phialide with endoconidia. × 1250.

Fig. 18. Phialide with twisted walls. Sabouraud's dextrose agar. × 600.

Fig. 19. Fructification like Corda's type. Sabouraud's dextrose agar. × 1250.

Fig. 20. Diagram showing a type of branching.

Fig. 21. Another type of branching.

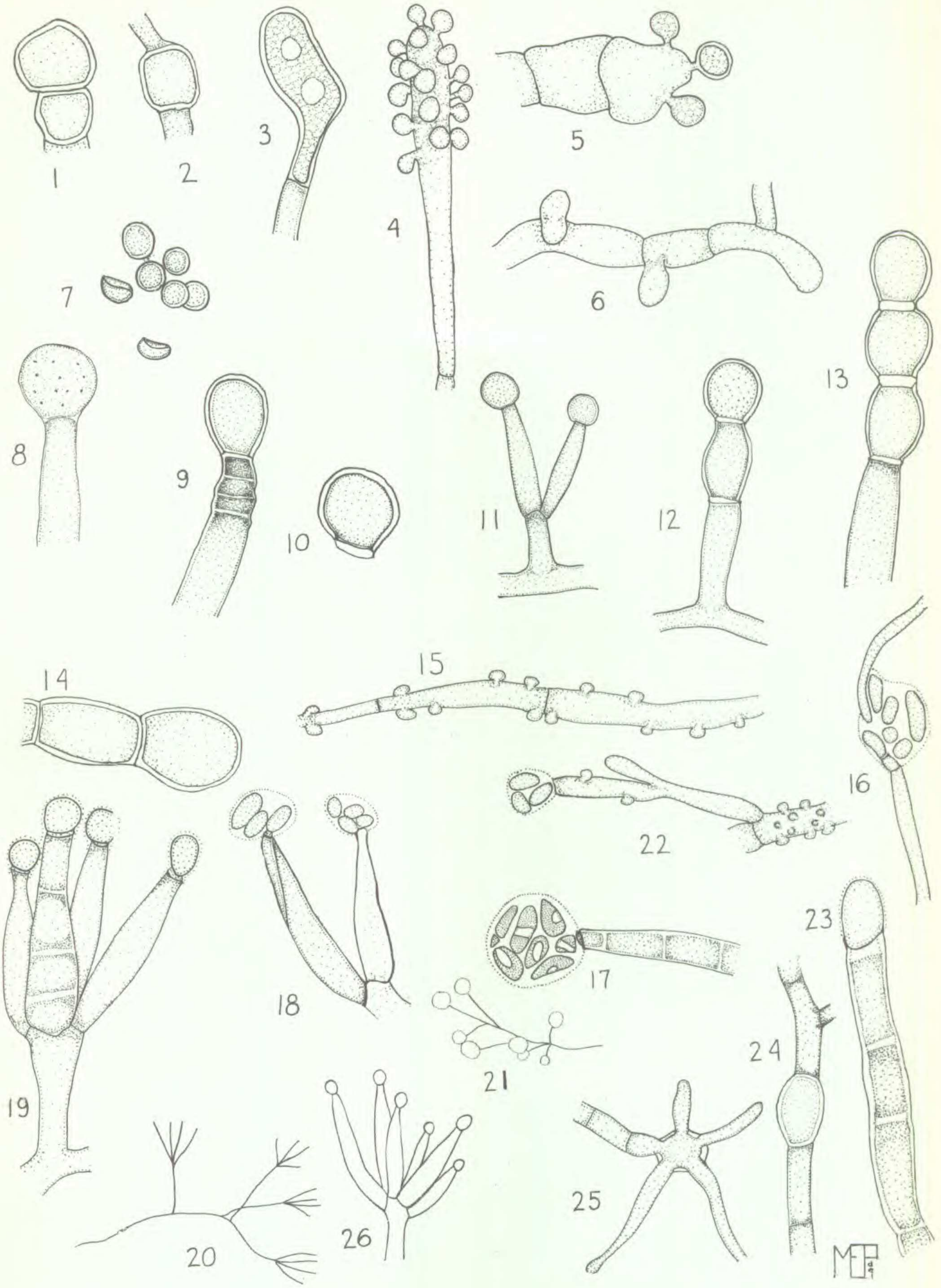
Fig. 22. Phialide with warts. Czapek's agar. × 350.

Fig. 23. Endoconidia. Sabouraud's dextrose agar. × 1250.

Fig. 24. Intercalary chlamydospore. Lactose broth. × 600.

Fig. 25. Germinating conidium. Lactose broth. × 600.

Fig. 26. Conidiophore with six concurrent branches. Wort agar. × 600.



PINKERTON — CONIDIAL FORMATION

COCKAYNE, BOSTON

Explanation of Plate

PLATE 6

Figs. 1–14, giant colonies on Czapek's agar.

Fig. 1. *Cephalosporium niveolanosum*
Fig. 2. *Cephalosporium symbioticum*
Fig. 3. *Gliocladium deliquescens*
Fig. 4. *Gliocladium fimbriatum*
Fig. 5. *Gliocladium penicilloides*
Fig. 6. *Gliocladium Vermoeseni*
Fig. 7. *Allescheria Boydii*
Fig. 8. *Clonostachys Araucaria* var. *confusa*
Fig. 9. *Cephalosporium rubrobrunneum*
Fig. 10. *Clonostachys Araucaria* var. *rosea*
Fig. 11. *Clonostachys Araucaria*
Fig. 12. *Cephalosporium kiliense*
Fig. 13. *Cephalosporium Serrae*
Fig. 14. *Cephalosporium Stuehmeri*

Figs. 15–18, photomicrographs.

Figs. 15–16. Granular incrustations of *Cephalosporium rubrobrunneum* upon blood serum.

Fig. 17. Branched conidiophore of *Clonostachys Araucaria* with warts and cephalosporia. × 1000.

Fig. 18. Agar micro-culture showing cephalosporia in *Cephalosporium symbioticum*. × 1000.